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**IMPROVING CHEMOTHERAPY IN ADVANCED  
UROTHELIAL CANCER: REAL-WORLD DATA  
STUDIES AND PROSPECTIVE CLINICAL TRIALS**

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# **Improving chemotherapy in advanced urothelial cancer: Real-world data studies and prospective clinical trials**

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To my family



## ABSTRACT

Advanced urothelial cancer (UC) is an aggressive disease with high morbidity and mortality. The primary aim of this thesis was to improve chemotherapy with respect to treatment efficacy, safety, and health-related quality of life (HRQoL) in advanced UC. The recently introduced chemotherapeutical drug vinflunine was investigated as monotherapy and in novel treatment combinations for metastatic UC (mUC). Further, two different neoadjuvant chemotherapy regimens were evaluated in muscle-invasive bladder cancer (MIBC).

Platinum-based combination chemotherapy have been standard treatment in mUC patients since the late 1980s. Vinflunine, approved in 2009, show a small but important improvement in survival when given as second line treatment. In **Paper I**, patients treated with vinflunine were evaluated in a retrospective real-world data study. The results confirmed that vinflunine is an active drug in an unselected cohort of routine patients. Further, patients with poor performance status had short survival and a high frequency of adverse events.

With the aim of further improving the efficacy of second-line treatment of mUC, vinflunine was combined with sorafenib in the dose-finding phase I trial VINSOR (**Paper II**). A recommended phase II dose could be identified. The adverse events generally agreed with those previously reported for vinflunine and sorafenib as monotherapy. The novel combination generated clinically meaningful disease stabilisation and tumour responses.

There is an unmet medical need for new treatment options for cisplatin-ineligible patients in first-line mUC, which was addressed in the randomised phase II trial VINGEM (**Paper III**) that compared the experimental combination of vinflunine and gemcitabine (VG) and standard treatment with gemcitabine and carboplatin. Compared to standard treatment, VG did not improve the primary endpoint progression-free survival. However, patients treated with VG did show a notably high overall response rate that was similar to the best data reported for any systemic therapy for mUC. The toxicity profile for VG was generally manageable, although high rates of neutropenia and febrile neutropenia were observed. No significant differences in HRQoL were found between the two treatment arms.

For patients with MIBC, neoadjuvant cisplatin-combination chemotherapy prior to cystectomy improves overall survival, but the optimal regimen is still unknown. A more cisplatin-dose-intense 3-week schedule was compared with a 4-week schedule of gemcitabine and cisplatin (GC) as neoadjuvant treatment in a retrospective study (**Paper IV**). Compared to the 4-week schedule, the 3-week schedule led to a significantly higher degree of pathological complete response, although this was associated with more frequent neutropenia. Despite the differences in downstaging, no differences in survival were observed between the two schedules.

In summary, vinflunine is an active drug for second-line treatment of mUC patients in a real-world setting. The novel combination of vinflunine and sorafenib can be safely combined in second-line treatment. In cisplatin-ineligible patients, compared to standard first-line treatment, the experimental combination of VG shows a higher response rate but does not prolong survival. In patients with MIBC, a more cisplatin-dose-intense 3-week schedule achieves significantly more complete responses compared to a 4-week schedule of GC as neoadjuvant chemotherapy prior to cystectomy.





## LIST OF SCIENTIFIC PAPERS

- I. **Karin Holmsten**, Line Hammer Dohn, Niels Viggo Jensen, Carl-Henrik Shah, Fredrik Jäderling, Helle Pappot and Anders Ullén. *Vinflunine treatment in patients with metastatic urothelial cancer: A Nordic retrospective multicenter analysis*. *Oncol Lett* 2016; 12: 1293-1300.
- II. Carl-Henrik Shah, Helle Pappot, Mads Agerbæk, **Karin Holmsten**, Fredrik Jäderling, Jeffrey Yachnin, Per Grybäck, Hans von der Maase and Anders Ullén. *Safety and activity of sorafenib in addition to vinflunine in post-platinum metastatic urothelial carcinoma (VINSOR): Phase I trial*. *Oncologist* 2019; 24: 745-e213.
- III. **Karin Holmsten**, Niels Viggo Jensen, Lene Sonne Mouritsen, Erika Jonsson, Camilla Mellnert, Mads Agerbæk, Cecilia Nilsson, Mette Moe, Andreas Carus, Elisabeth Öfverholm, Outi Lahdenperä, Yvonne Brandberg, Hemming Johansson, Mats Hellström, Hans von der Maase, Helle Pappot and Anders Ullén. *Vinflunine/gemcitabine versus carboplatin/gemcitabine as first-line treatment in cisplatin-ineligible patients with advanced urothelial carcinoma: A randomised phase II trial (VINGEM)*. *Eur J Cancer* 2020; 127: 173-182.
- IV. **Karin Holmsten**, Lise Høj Omland, Anne Birgitte Als, Mads Agerbæk, Line Hammer Dohn, Henriette Lindberg, Niels Viggo Jensen, Andreas Carus, Mette Moe, Abolfazl Hosseini, Cecilia Radkiewicz, Helle Pappot and Anders Ullén. *Three-week versus four-week schedule of neoadjuvant gemcitabine and cisplatin in muscle-invasive bladder cancer: Implications for efficacy and safety*. Manuscript submitted.



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## LIST OF ABBREVIATIONS

ADC	antibody-drug conjugate
AE	adverse event
BSC	best supportive care
CI	confidence interval
CR	complete response
DCR	disease control rate
DDR	DNA damage repair
DLT	dose-limiting toxicity
ECOG PS	Eastern Cooperative Oncology Group performance status
eCRF	electronic case report form
EMA	European Medicines Agency
EORTC	European Organisation for Research and Treatment of Cancer
FDA	Food and Drug Administration
FGFR	fibroblast growth factor receptor
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GFR	glomerular filtration rate
HR	hazard ratio
HRQoL	health-related quality of life
ICI	immune checkpoint inhibitor
irAE	immune-related adverse event
ITT	intention-to-treat
MIBC	muscle-invasive bladder cancer
mOS	median overall survival
mPFS	median progression-free survival
MTD	maximum tolerated dose
mUC	locally unresectable and metastatic urothelial cancer
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events

NUCOG	Nordic Urothelial Cancer Oncology Group
OR	odds ratio
ORR	overall response rate
OS	overall survival
PD	progressive disease
PD-L1	programmed death-ligand 1
PFS	progression-free survival
PR	partial response
PRO	patient-reported outcome
PS	performance status
QLQ-C30	Quality of Life Questionnaire Core 30
RECIST	Response Evaluation Criteria in Solid Tumours
RPTD	recommended phase II dose
RWD	real-world data
SAE	serious adverse event
SD	stable disease
SUSAR	suspected unexpected serious adverse reaction
TNM	tumour, (regional lymph) nodes, (distant) metastases
TUR-B	transurethral resection of the bladder
UC	urothelial cancer
VEGFR	vascular endothelial growth factor receptor
WHO	World Health Organization

## LIST OF ABBREVIATIONS OF CHEMOTHERAPY REGIMENS

dd-GC	dose-dense gemcitabine and cisplatin
dd-MVAC	dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin
GC	gemcitabine and cisplatin
GCa	gemcitabine and carboplatin
GC-3w	gemcitabine and cisplatin 3-week schedule
GC-4w	gemcitabine and cisplatin 4-week schedule
HD-MVAC	high-dose methotrexate, vinblastine, doxorubicin, and cisplatin
M-CAVI	methotrexate, carboplatin, and vinblastine
MVAC	methotrexate, vinblastine, doxorubicin, and cisplatin
PCG	paclitaxel, cisplatin, and gemcitabine
VCa	vinflunine and carboplatin
VG	vinflunine and gemcitabine





# 1 BACKGROUND

## 1.1 INTRODUCTION TO ADVANCED UROTHELIAL CANCER

Urothelial cancer (UC) is a major global health concern and ranges from superficial non-muscle-invasive tumours with good prognosis to muscle-invasive tumours of the bladder and deadly metastatic disease. This dissertation is focused on advanced UC, defined as muscle-invasive bladder cancer (MIBC) and metastatic urothelial cancer (mUC), the latter of which also includes locally unresectable tumours. Advanced UC is a highly aggressive disease associated with substantial morbidity and mortality [1, 2]. There is a need to improve medical treatment, including chemotherapy, for patients with MIBC and mUC in order to increase survival, decrease treatment-related side effects, and improve quality of life. An overview of the current treatment options in MIBC and mUC is presented in Figure 1.

The standard treatment for patients with MIBC consists of cisplatin-based neoadjuvant chemotherapy followed by radical cystectomy. Despite intense treatment, the median 5-year survival is only about 50% [1, 2], mainly due to treatment failure and development of distant metastases.

Metastatic UC is a lethal disease with an overall survival (OS) of only roughly 6 months without treatment and approximately 15 months with the best treatment options available today [1, 2]. For patients with mUC, platinum-based combination chemotherapy has been the standard treatment since the late 1980s, displaying a high overall response rate (ORR) [3]. However, the duration of the responses is generally short, and adverse events are a common clinical challenge. Vinflunine was approved as second-line treatment in 2009, and has led to a small but important improvement in the survival of patients with mUC [4]. The development of immunotherapy has been a major breakthrough in many cancer diagnoses, as well for treatment of UC [5]. However, the response rate to immunotherapy in mUC is rather low, emphasising the importance of chemotherapy as a cornerstone in the treatment of this severely ill patient population.

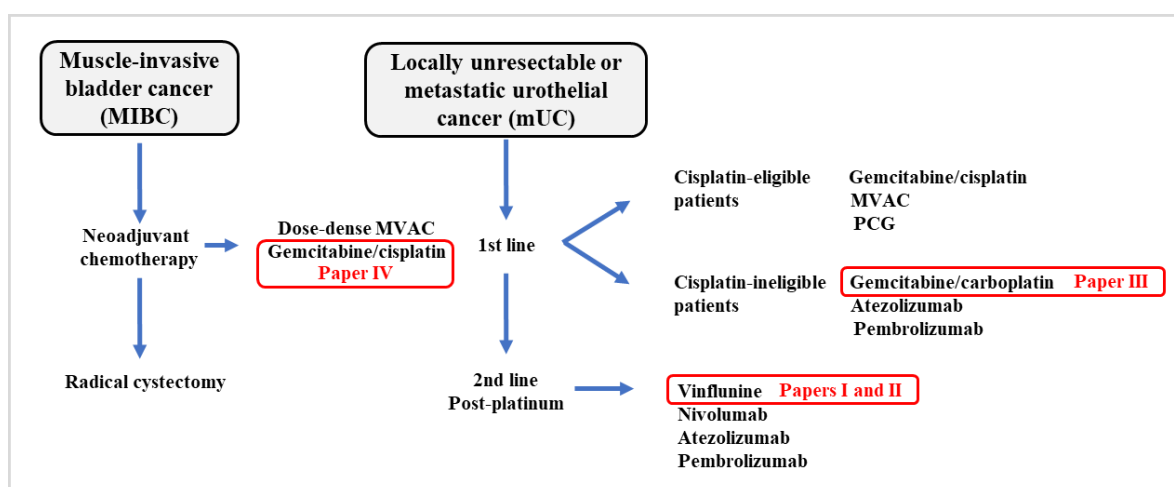


Figure 1. Schematic overview of the present treatment options for patients with MIBC and mUC. MVAC, methotrexate, vinblastine, doxorubicin and cisplatin; PCG, paclitaxel, cisplatin and gemcitabine.

The following sections present a literature review focused on chemotherapy treatment of MIBC and mUC patients with the aim of providing the background and rationale for performing the four studies included in this thesis.

### **1.1.1 Epidemiology and etiology**

UC consists of primary cancers from the urinary collection systems originating in the urinary bladder (approximately 95% of the cases), renal pelvis, ureter and urethra [1]. The majority of UCs arise from the urothelium (transitional epithelial cells) lining the mucous membranes of the urinary tract. About two thirds of UC cases are non-muscle invasive, and approximately one third are muscle invasive and locally unresectable or primary metastatic at diagnosis.

UC is a common cancer associated with high mortality. The global incidence is about 550 000 cases annually [6], and UC is the twelfth most common cancer worldwide. In Sweden, UC is the seventh most frequent cancer, with 3402 new cases in 2018 [7]. Mortality in UC is high at an annual rate of 200 000 deaths globally and around 750 deaths in Sweden. According to the Swedish National Registry of Urinary Bladder Cancer (SNRUBC), approximately 23% of the cases in Sweden are muscle invasive at diagnosis [8].

The aetiology of UC is multifactorial and not fully known. Age and sex are important risk factors [7, 9], with a mean age of 70 years at diagnosis and a male-to-female ratio of 2–3:1. The most common external risk factor for UC is tobacco, and it is estimated that approximately 50% of the cases are associated with cigarette smoking [10]. Exposure to certain chemicals, mainly aromatic amines, is also an important risk factor for UC [11]. Radiotherapy to the pelvis region and specific chemotherapy (e.g., cyclophosphamide, melphalan, and thiotepa) may cause secondary malignancy in the urothelial tract [12-14]. Infection with the parasite *Schistosoma* is distinctly associated with an increased risk of squamous cell carcinoma of the bladder [15], and such infection is common in developing countries, particularly in Africa and the Middle East.

### **1.1.2 Clinical presentation and classification**

Haematuria is the most typical alarm symptom for UC and should be investigated rapidly. Approximately one out of three patients with macroscopic haematuria are diagnosed with a tumour in the urinary tract or in the prostate, and about 75% of patients with UC experience haematuria at the time of diagnosis [16]. For patients with locally advanced disease, growth of the tumour in the pelvis can cause severe symptoms such as pain, haematuria, hydronephrosis, and renal failure [1, 2]. Regional lymph node metastases in the abdomen and pelvis are common. Distant metastases are most often located in the lungs, liver, bone, and central nervous system.

UC is diagnosed through cystoscopy, transurethral resection of the bladder (TUR-B), histopathological assessment of the tumour lesion, and radiological imaging [1, 2]. Bladder cancer is staged according to the TNM classification [17] and is divided into two main groups: non-muscle-invasive tumours (Ta, CIS, and T1) and muscle-invasive tumours (T2–T4N0M0).

Computerised tomography (CT) and magnetic resonance imaging (MRI) are the diagnostic radiological methods most frequently used to stage the primary tumour, the regional lymph nodes, and distant metastases (together designated TNM) [1, 2]. However, correlation is low between the preoperative clinical (i.e., radiological) T stage and the pathological stage after cystectomy. Some studies have shown accuracy to be as low as 50%, where CT tends to underestimate [18] and MRI overestimate [19] the T stage. Histopathological assessment provides the tumour grade according to the World Health Organization (WHO) classification of tumours of the urinary system 2016 [20].

### **1.1.3 Molecular biology**

In recent years, extensive research has increased the knowledge regarding the molecular biology of UC [21-23]. It is postulated that UC develops along two distinct molecular pathways: papillary non-muscle-invasive UC that has a good prognosis, and non-papillary genetically unstable MIBC that arises from carcinoma in situ and has a poor prognosis [24]. Nevertheless, UC shows wide heterogeneity in molecular features that are independent from conventional staging such as TNM stage and WHO grade [23].

Molecular classification of UC has been established suggesting three major molecular subtypes, luminal, basal and neuroendocrine differentiation, with several possible subgroups [25]. The subtypes are characterized by different alterations in important tumor biology hallmarks, e.g. cell-cycle regulation, immune signaling, tyrosine receptor kinases pathways, cytokeratin alterations, cell adhesion mechanisms and mutations in FGFR3, PI3KCA and p53. Furthermore, molecular subtypes show distinct clinical outcomes, e.g. patients with the subgroup “luminal papillary” show good prognosis and patients with basal differentiation poor prognosis [25, 26].

## **1.2 SYSTEMIC TREATMENT IN MUSCLE INVASIVE BLADDER CANCER (MIBC)**

Radical cystectomy is the standard curative treatment for patients with MIBC, but the relapse rate is high. The 5-year recurrence-free survival is reported to be 30–52% [2, 27], with local recurrences arising in 5–15% of the patients and metastatic disease in 20–50% [2, 27, 28]. External beam radiotherapy with concomitant chemotherapy is an alternative curative-intent treatment for MIBC, and it offers local control and survival rates comparable to radical cystectomy [29]. About 50% of the patients with MIBC are considered ineligible for curative therapies due to high age, poor performance status (PS), or comorbidities [8, 30]. Thus, there is a great need for improved treatment strategies for patients with MIBC.

### **1.2.1 Neoadjuvant treatment**

In MIBC, neoadjuvant cisplatin-based chemotherapy improves OS compared to radical cystectomy alone. The purpose of preoperative chemotherapy is to reduce the primary tumour in the bladder and to eradicate micrometastases. In the 1980s and 1990s, neoadjuvant cisplatin-

based chemotherapy was investigated in several randomised trials. Three large meta-analyses concluded that neoadjuvant treatment reduced the risk of death by 10–14% and led to a 5% increase in absolute median overall survival (mOS) at 5 years in comparison with cystectomy alone [31–33]. The cited trials were heterogeneous in terms of patient characteristics, type of cisplatin combinations, and primary therapy (cystectomy and/or radiotherapy). The different cisplatin-based regimens have not been investigated head-to-head in randomised clinical trials. For patients ineligible for cisplatin due to impaired renal function, poor PS, or comorbidities, there is no evidence-based treatment available for use in the neoadjuvant setting [34]. The combination methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) has been studied in a randomised phase III trial that showed a significant 5-year mOS advantage compared to cystectomy alone (57% vs 43%) [35], and MVAC is commonly used as a neoadjuvant regimen. The combination of gemcitabine and cisplatin (GC) is also frequently used in the neoadjuvant setting based on the results of a randomised phase III mUC trial in which GC and MVAC had similar response rates and survival outcomes, but GC had a more favourable toxicity profile [36]. No neoadjuvant randomised phase III trials with GC have yet been performed.

A large number of retrospective real-world data (RWD) studies have been performed to address the efficacy of MVAC and GC as neoadjuvant treatment in patients with MIBC. The largest investigation thus far included 785 patients, and it indicated that MVAC and GC had similar pathological complete response rates (pT0N0) of approximately 25%, and similar partial downstaging (< pT2N0) of approximately 45%, along with no significant difference in survival [37]. Pooled data in meta-analyses summarise pathological response rates (pT0N0 and < pT2N0) and survival in the same range for GC as for MVAC [38–41]. Dose-dense (dd) regimens in combination with granulocyte colony-stimulating factor (G-CSF) have been explored, and show promising downstaging rates and tolerable toxicity profiles (dd-MVAC [42–45] and dd-GC [46, 47]). To a large extent, dd-MVAC has replaced conventional MVAC as neoadjuvant treatment in clinical practice [1]. Different doses and numbers of cycles of MVAC, dd-MVAC, and GC have been used, but the optimal neoadjuvant regimen is not yet known. For GC, the most commonly used neoadjuvant schedule is either four cycles of a more cisplatin-dose-intense 3-week schedule or three cycles of a commonly applied 4-week schedule. In mUC, these two GC schedules appear to be similarly efficient, although with less haematological toxicity in the 3-week schedule [48]. In the neoadjuvant setting, the preferable GC schedule has not been determined. The aim of the study reported in **Paper IV** was to compare the 3-week and the 4-week schedule of GC as neoadjuvant treatment in patients with MIBC with respect to treatment patterns, toxicity, downstaging efficacy, and survival.

Pathological complete response (pT0N0) at cystectomy after neoadjuvant chemotherapy in patients with MIBC is suggested to be a surrogate marker for the overall treatment efficacy. Patients with pT0N0 have a significantly better prognosis than patients without complete downstaging, showing 5-year survival rates of approximately 85–90% and 50%, respectively [35, 49, 50]. Rosenblatt *et al.* [49] demonstrated increased survival of approximately 30% in patients who achieved complete response due to neoadjuvant chemotherapy compared to those

who achieved pT0 after TUR-B only, which illustrates the importance of treating possible micrometastases.

Besides chemotherapy, immunotherapy with immune checkpoint inhibitors (ICIs) are presently being explored in the neoadjuvant setting. Monotherapy with pembrolizumab and with atezolizumab provided promising results in single phase II trials with pT0N0 of 42% and 31%, respectively, in patients with MIBC [51, 52]. Several randomised neoadjuvant phase II and III trials including ICIs as monotherapy or in combination with chemotherapy are ongoing, but as of yet no data are available from these studies [5, 34].

### 1.2.2 Adjuvant treatment

Adjuvant chemotherapy after radical cystectomy in patients with MIBC has been evaluated in several trials, although the findings are inconsistent with regard to potential survival advantages. Two meta-analyses indicated a 25% reduction in mortality and a 9% increase in OS at 3 years, but the trials included in those meta-analyses were criticised for small sample sizes, flaws in trial design, and statistical considerations [53, 54]. A phase III trial comparing immediate versus delayed (at time of visible relapse) chemotherapy for patients with residual tumour at cystectomy (pT3–pT4 or node-positive disease) did not show any survival advantage for immediate compared to delayed treatment, although an increase in 5-year median progression-free survival (mPFS) was observed [55]; unfortunately, this trial had low statistical power. In conclusion, adjuvant chemotherapy is not generally recommended as standard treatment after cystectomy due to lack of evidence, but it can be considered in selected patients with advanced or node-positive disease who have not received neoadjuvant chemotherapy [1, 2].

## 1.3 SYSTEMIC TREATMENT IN METASTATIC UROTHELIAL CANCER (mUC)

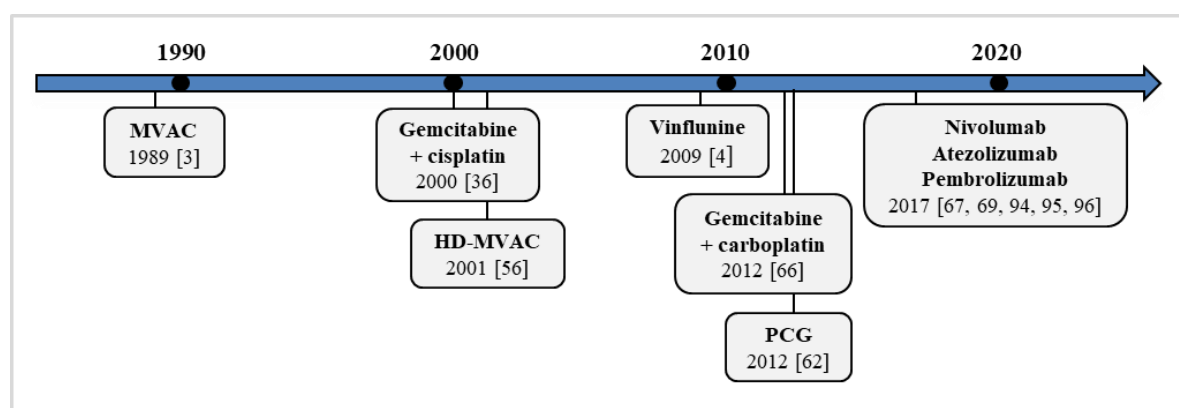


Figure 2. Timeline of the positive landmark mUC trials.  
MVAC, methotrexate, vinblastine, doxorubicin and cisplatin; PCG, paclitaxel, cisplatin and gemcitabine.

### 1.3.1 First-line treatment

The standard treatment for non-curable locally unresectable (cT4b and/or N1-3) or metastasised UC has been platinum-based combination-chemotherapy since the late 1980s. In

1989, a trial with MVAC showed an impressive ORR of 72% and mOS of 14.8 months [3]. However, MVAC is associated with considerable toxicity: 3% drug-related deaths, 25% neutropenic fever, 58% myelosuppression, and 49% mucositis. With high-dose MVAC (HD-MVAC), a dose of cisplatin that is twice as high as in classic MVAC is given together with G-CSF [56, 57]. Compared to MVAC, HD-MVAC induces a significantly higher ORR (64% vs 50%) but no significant increase in mOS (15.1 vs 14.9 months). Also, due to the addition of G-CSF, HD-MVAC causes less neutropenia and neutropenic fever compared to MVAC.

A randomised phase III trial, presented in 2000, compared GC with MVAC and demonstrated no significant difference in either ORR (46% vs 49%) or mOS (13.8 vs 14.8 months) but did show a better safety profile for GC [36, 58]. The patients in the GC arm in comparison with the subjects given MVAC displayed significantly less grade 3/4 neutropenia (71% vs 82%), neutropenic fever (3% vs 26%), and grade 3/4 mucositis (1% vs 22%). Therefore, due to the more favourable safety profile, GC has to a large extent replaced MVAC as the standard first-line treatment in mUC [1, 2].

The taxanes paclitaxel and docetaxel have been investigated in single, doublet, and triplet regimens for mUC [59]. Doublet regimens combining a taxane and a platinum agent (docetaxel/cisplatin [60] and paclitaxel/carboplatin [61]) have induced similar or inferior responses and survival compared to MVAC. Various triplet regimens have been investigated in single-arm phase II trials with promisingly high response rates and OS [59]. However, only one randomised phase III trial including three chemotherapeutic drugs for mUC has been completed, and it compared GC alone with GC plus paclitaxel (PCG) [62]. Adding paclitaxel to GC did not significantly improve the mOS compared to GC alone (15.8 months vs. 12.7 months), although it did result in a significant increase in ORR (56% vs 44%). It should also be noted that a higher degree of neutropenic fever was seen in the triplet arm PCG.

### **1.3.2 First-line treatment in cisplatin-ineligible patients**

Approximately half of all patients with mUC are ineligible (“unfit”) for cisplatin-based chemotherapy due to impaired renal function, poor PS, co-morbidities (e.g., hearing loss, peripheral neuropathy, or heart failure), or high age [63]. Being unfit in this context is commonly defined as having an Eastern Cooperative Oncology Group performance status (ECOG PS) of  $\geq 2$  and/or a glomerular filtration rate (GFR) of  $< 60$  ml/min [64]. Several regimens for treatment of the unfit population have been investigated in multiple small single-arm phase II trials, including monotherapy and combinations of gemcitabine, carboplatin, paclitaxel, oxaliplatin, vinblastine, and epirubicin [63].

Only one randomised phase III trial has been reported for patients ineligible for cisplatin and that trial compared gemcitabine and carboplatin (GCa) and methotrexate, carboplatin, and vinblastine (M-CAVI) [65, 66]. This study showed no significant differences between the two treatment arms in ORR (41% vs 30%) or in mOS (9.3 vs 8.1 months), although GCa showed a significant favourable toxicity profile (grade 3/4 mucositis, neutropenic fever, renal toxicity, and grade 4 thrombocytopenia associated with bleeding and death), 9% for GCa vs 21% for

M-CAVI. Patients with both PS  $\geq 2$  and impaired renal function had shorter mOS, lower ORR, and a higher degree of severe acute toxicity. Based on the results of this trial, in most countries GCa is considered as the standard first-line treatment for mUC patients unfit for cisplatin [1, 2].

In 2017, immunotherapy with ICIs was approved for first-line treatment of cisplatin-unfit patients. In single-arm phase II trials, pembrolizumab [67, 68] and atezolizumab [69], respectively, resulted in ORRs of 24% and 23%, and mOS of 11.3 and 15.9 months. The toxicity profile for immunotherapy was generally favourable compared to carboplatin-based chemotherapy. The most widely reported adverse events (AEs) associated with pembrolizumab and atezolizumab were fatigue (18% and 30%), diarrhoea (12%), and pruritus (11% and 18%). Decreased renal function occurred in only about 4% of the studied subjects, thus ICIs are suitable options for patients that are ineligible for cisplatin treatment due to impaired renal function. The efficacy of ICIs varies with the level of expressed programmed death-ligand 1 receptor (PD-L1), and only approximately 30% of patients with mUC express high levels of PD-L1 [70]. Today, the use of pembrolizumab and atezolizumab as first line treatment in mUC patients unfit for cisplatin is restricted to individuals with high expression of PD-L1 [71, 72].

In 2009 vinflunine was approved as second-line treatment after platinum-based chemotherapy for patients with mUC, and since then this drug has been further explored in combination with gemcitabine and carboplatin as first-line treatment for cisplatin-ineligible patients. The safety of the combinations vinflunine and gemcitabine (VG) and vinflunine and carboplatin (VCa) were initially assessed in phase I trials [73, 74]. The randomised phase II trial JASINT1 compared VG and VCa, and showed promising response rates and survival, although no statistical difference was seen between the two groups (ORR 44% for VG and 29% for VCa, mOS 14.0 months for VG and 12.8 months for VCa) [75]. A more favourable toxicity profile with less grade 3/4 haematological AEs was noted for the VG arm compared to the VCa arm. The main criticism of the JASINT1-trial has been that it did not include a non-investigational control arm such as GCa.

Patients with mUC that are ineligible for cisplatin represent a fragile population with inferior survival compared to patients that can receive cisplatin combination therapy. Although no phase III trial has been conducted to compare cisplatin and carboplatin-based regimens head-to-head for treatment of mUC, small randomised phase II trials have indicated superiority of cisplatin combinations [76-78]. Furthermore, retrospective RWD show a significantly longer survival for patients treated with cisplatin-based chemotherapy compared to those given carboplatin-based combinations or non-platinum regimens [79, 80]. There is clearly a need to develop more effective novel regimens for the cisplatin-ineligible population. To address the need for new treatment options for cisplatin-unfit patients in first-line mUC, the randomised phase II trial VINGEM (**Paper III**) compared treatment with experimental VG with standard GCa.

### 1.3.3 Second-line treatment and beyond

Patients with mUC that progress on first-line platinum chemotherapy represent a heterogeneous population. Some patients present with good PS and slowly progressing limited disease, whereas others display large-volume disease that affects the function of vital organs and causes a high degree of comorbidities, poor overall PS, and short survival. The patients in this fragile cohort tend to benefit less from chemotherapies due to an increased risk of more severe side effects, which also limits the feasibility of and patient accrual in randomised trials.

Numerous small single-arm phase II trials have investigated different chemotherapy drugs in second-line, both as monotherapy and in combinations. Non-platinum drugs such as cabazitaxel, docetaxel, ifosfamide, irinotecan, paclitaxel, nab-paclitaxel, and pemetrexed have resulted in ORRs of 5–29% [81] and modest mOS data of 5–10 months. Combination treatment tends to give higher response rates but has failed to translate this into prolonged survival [82].

As already stated, in 2009 vinflunine was the first drug to be approved as second-line treatment for patients with mUC. Vinflunine (Javlor®) is a novel microtubule inhibitor with higher levels of *in vitro* and *in vivo* tumour activity compared to other vinca alkaloids [83]. The efficacy of vinflunine was analysed in two phase II trials [84, 85] and further in a randomised phase III trial comparing vinflunine plus best supportive care (BSC) to BSC alone [4]. The mOS was significantly improved by 2.6 months (6.9 months for vinflunine vs 4.3 months for BSC) in the eligible population but not in the intention-to-treat (ITT) population [4, 86]. Vinflunine has been approved by the European Medicines Agency (EMA), but it has not been approved by the United States Food and Drug Administration (FDA) based on the ambiguity of the statistics in the ITT and eligible populations. ORR, disease control rate (DCR), mPFS, and multivariate analyses adjusting for prognostic factors all showed statistical significance in favour of vinflunine compared to BSC. The most frequent side effects of vinflunine were neutropenia (50%), anaemia (19%), febrile neutropenia (6%), fatigue (19%), and constipation (16%) [4]. At the time of commence of this dissertation, vinflunine had recently been introduced in routine clinical practice, although no RWD studies had been performed that validated the efficacy and safety of this drug in an unselected clinical cohort, and this issue is addressed in **Paper I**.

One strategy to further improve the outcome for patients progressing on first-line platinum treatment is to use vinflunine as a backbone in combination with other drugs. However, phase I trials studying vinflunine in combination with the antiangiogenic tyrosine kinase inhibitor pazopanib [87] and in combination with the antimetabolite chemotherapeutic drug pemetrexed [88] demonstrated that both those combinations were too toxic for further evaluation. It has been hypothesised that the antiangiogenic vascular endothelial growth factor receptor (VEGFR) multi-tyrosine kinase inhibitor sorafenib (Nexavar®) [89] might be effective for treatment of UC due to altered signalling in angiogenic pathways in UC tumours [90], and treatment with sorafenib has been investigated in small single-arm trials [91, 92] and has also been described in a case report with favourable outcome in one mUC patient [93]. Accordingly, the dose-finding phase I trial entitled VINSOR was initiated to assess the safety



of the combination of vinflunine and sorafenib, with the long-term aim of improving vinflunine treatment in patients with mUC (**Paper II**).

Immunotherapy with ICIs is a major breakthrough in mUC. Five ICIs have been approved by the FDA for use as second-line treatment in platinum-progressive mUC patients: atezolizumab [94], pembrolizumab [95], nivolumab [96], avelumab [97], and durvalumab [98]. In Sweden, atezolizumab, pembrolizumab, and nivolumab have been approved. Two randomised phase III trials [97, 98] evaluated pembrolizumab and atezolizumab compared with the investigators' choice of chemotherapy (vinflunine, paclitaxel, or docetaxel). The trial with pembrolizumab led to significantly prolonged mOS compared to chemotherapy (10.3 vs 7.4 months) [95], whereas the atezolizumab trial did not meet the primary endpoint of improved survival [94]. The ORR for ICIs in second-line treatment is generally rather low at 13–21%, and the mOS is between 8.6 and 18.2 months [99]. However, in patients that do respond to ICIs, duration of the response is long. For example, in the phase III trial with pembrolizumab, among those patients who did show a response, the response was still ongoing at 12 months in 68% in the pembrolizumab group compared to 35% in the chemotherapy group [95]. In the two phase III trials, pembrolizumab and atezolizumab showed a better safety profile with significantly lower incidence of grade 3/4 AEs compared to the control chemotherapy (15% for pembrolizumab and 20% for atezolizumab, and approximately 45% for chemotherapy) [94, 95]. For pembrolizumab, the most common side effects of all grades were pruritus (20%), fatigue (14%), and diarrhoea (9%), and the most frequent immune-related AEs (irAEs) were hypothyroidism (6%), pneumonitis (4%), hyperthyroidism (4%), and colitis (2%) [95].

For patients that progress on platinum chemotherapy, immunotherapy, and vinflunine, no evidence-based treatment options have yet been approved. These patients usually have an advanced disease with severe clinical symptoms, poor PS, and short expected survival. If eligible for treatment, paclitaxel and gemcitabine can be an option [100, 101], but these patients should preferably be included in clinical trials [1, 2].

## **1.4 PROGNOSTIC CLINICAL FACTORS AND MOLECULAR MARKERS**

Patients with advanced UC show large inter-individual differences in their responses to the available treatments. Prognostic and predictive factors for tumour response and survival are essential to individualise the treatment and to improve efficacy outcomes. Such factors can also enable stratification of patients in clinical trials [102].

Two negative clinical prognostic factors are well established in first-line treatment with platinum in mUC, namely the Bajorin factors: performance status (Karnofsky PS  $\leq$  80% or ECOG PS  $\geq$  1) and the presence of visceral metastases [103]. Bajorin *et al.* showed that the mOS after treatment with MVAC differs between 9.1 and 33.0 months depending on the number of prognostic factors (see Figure 3a). These two potential prognostic factors have been

confirmed for both cisplatin-eligible and cisplatin-ineligible populations in several large trials using different platinum regimens [58, 66, 104].

For second-line treatment in mUC, it has been suggested that ECOG PS  $\geq 1$ , the presence of liver metastasis, and low levels of haemoglobin (Hb < 10 g/dl) (referred to as the Bellmunt factors) are unfavourable prognostic markers [105]. In second-line treatment with vinflunine, the mOS differs from 1.7 to 14.2 months for patients with all three versus none of the suggested prognostic risk factors (see Figure 3b) [105].

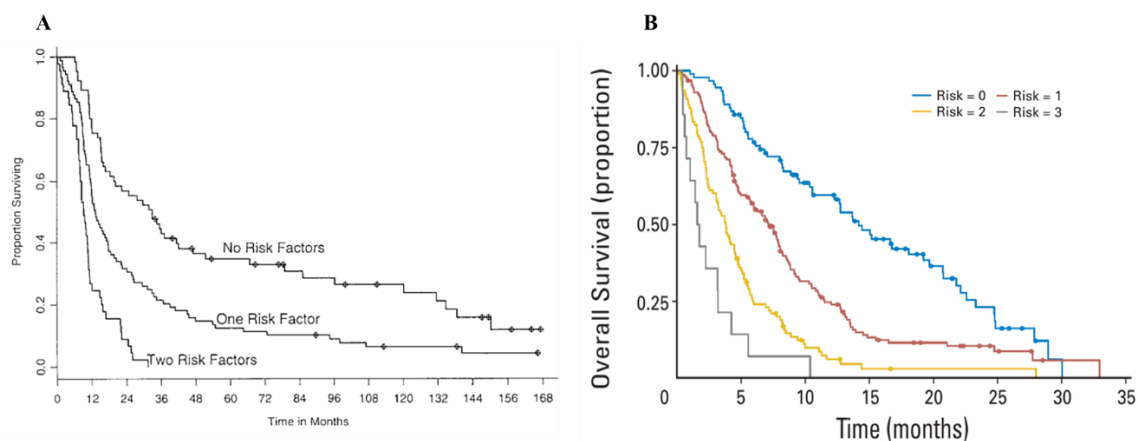


Figure 3. Kaplan-Meier curves displaying different number of risk factors in first-line treatment with MVAC (Bajorin *et al.* 1999) (A) and second-line treatment with vinflunine (Bellmunt *et al.* 2010) (B). Reprinted with premission. © (1999 and 2010) American Society of Clinical Oncology. All rights reserved.

There is further considerable heterogeneity in advanced UC with regard to *de novo* and acquired tumour resistance to the available systemic treatments. Molecular markers have the potential to personalise oncological treatment. Some promising candidate markers have been identified, but there are presently no biomarkers in use in clinical practice. Progress is being made in establishing a molecular classification for UC, which may prove to be a useful predictive molecular marker for different systemic treatments in the different stages of the UC disease [106, 107]. Alterations in DNA damage repair (DDR) genes may affect the response to platinum treatment and can potentially be valuable for analysis of patients with MIBC in the neoadjuvant setting, as well as to select chemotherapy or immunotherapy for patients with mUC [107, 108]. For immunotherapy, expression of PD-L1, mutations in DDR genes, tumour mutational burden, and microsatellite instability are possible molecular markers [109, 110]. Molecular markers and individualised treatment are addressed further in the Discussion (Chapter 5).

## 1.5 ADVERSE EVENTS AND HEALTH-RELATED QUALITY OF LIFE

Patients with mUC are often fragile due to severe disease-associated symptoms, comorbidity, and high average age, and thus they are susceptible to treatment-related toxicity. All of the widely used chemotherapeutic drugs for UC (cisplatin, carboplatin, gemcitabine, methotrexate, vinblastine, doxorubicin, paclitaxel, and vinflunine) are to different extents

associated with potentially severe side effects, as described above. Most common is haematological toxicity, which can be potentially dangerous or even fatal due to neutropenic sepsis or bleeding secondary to thrombocytopenia. Other common side effects that have a great impact on HRQoL are fatigue, nausea and vomiting, and loss of appetite, and for vinflunine also constipation and stomach pain. In general, ICIs have a more favourable toxicity profile than chemotherapy and therefore may be somewhat safer to administer in older patients and patients with poor PS. However, even if grade 3/4 AEs are less frequent with ICIs (approximately 10–20%) compared with chemotherapy, ICIs are also associated with the risk of irAEs (e.g., pneumonitis, thyroiditis, and colitis) that can be severe and even lethal [111].

In addition to assessing the toxicity profile related to treatment, it is important to measure the patients' HRQoL. Historically, research on HRQoL in UC has focused primarily on surgical procedures in MIBC patients, including complications of different urinary diversion techniques [112]. Data are more limited regarding HRQoL in patients with chemotherapy or other systemic medical treatments for MIBC and mUC [113]. For patients with MIBC, HRQoL seems to be inversely related to sexual function and urinary tract symptoms and increase six months after treatment. The impact of the neoadjuvant chemotherapy on HRQoL, however, is not studied separately from the influence of cystectomy [113]. In two large randomized clinical trials with first-line platinum chemotherapy in patients with mUC, HRQoL seemed to be stable or improve during the palliative chemotherapy compared to baseline measurements [36, 66], however the analyses were associated with low compliance and absence of bladder specific questionnaires. No studies have reported HRQoL data in RWD studies outside clinical trials for patients with MIBC or mUC [113]. The European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire Core 30 (QLQ-C30) has been developed to assess the quality of life in cancer patients [114] and is the core instrument used in clinical trials in oncology to measure global health status, functioning (physical, emotional, cognitive and social), and common cancer-related symptoms [115]. At the onset of the present studies, no diagnosis-specific questionnaire had yet been developed for urothelial cancer.

## 2 AIMS

The primary aim of this thesis was to improve chemotherapy in advanced urothelial cancer by investigating the recently introduced chemotherapeutic drug vinflunine in monotherapy and in novel treatment combinations, and to evaluate two different neoadjuvant chemotherapy regimens, with the overall goal of increasing survival and improving health-related quality of life (HRQoL).

The specific aims of the present studies were as follows:

### Paper I

- To evaluate treatment patterns, response rates, survival parameters, and adverse events of the novel vinca alkaloid vinflunine in patients with second-line metastatic urothelial cancer (mUC) in a routine clinical cohort (retrospective real-world data [RWD] study).
- To investigate clinical prognostic factors for second-line treatment in mUC.

### Paper II

- To examine safety and establish a recommended phase II dose for the novel combination of vinflunine and the antiangiogenic tyrosine kinase inhibitor sorafenib in second-line mUC patients (prospective dose-finding phase I trial).
- To assess anti-tumour efficacy of vinflunine in combination with sorafenib.

### Paper III

- To compare progression-free survival of treatment with the experimental combination of vinflunine and gemcitabine versus standard treatment with carboplatin and gemcitabine in first-line cisplatin-ineligible mUC patients (prospective randomised phase II trial).
- To analyse response rates, overall survival, toxicity, and HRQoL in the experimental and control arms.

### Paper IV

- To compare the pathological downstaging response to two different schedules of gemcitabine and cisplatin, a more cisplatin-dose-intense 3-week versus a 4-week schedule, as neoadjuvant chemotherapy prior to cystectomy in muscle-invasive bladder cancer (retrospective RWD study).
- To examine the implications for anti-tumour efficacy, survival, treatment patterns, and safety.

### 3 PATIENTS, MATERIALS, AND METHODS

An overview of the patients, materials, and methods in the four papers included in this thesis is presented in Table 1.

**Table 1.**

**Summary of patients, materials, and methods in the papers included this thesis**

Paper	I	II	III	IV
<b>Design</b>	Retrospective RWD	Dose-finding clinical phase I trial, 3+3 dose escalation	Randomised controlled phase II screening trial	Retrospective RWD
<b>Study group</b>	Second-line mUC, 100 patients at 3 centres in Sweden and Denmark	Second-line mUC, 22 patients at 3 centres in Sweden and Denmark	First-line mUC cisplatin-ineligible, 62 patients at 11 centres in Sweden, Denmark, and Finland	MIBC, 706 patients at 7 centres in Sweden and Denmark
<b>Treatment drugs</b>	Vinflunine	Vinflunine + sorafenib	Vinflunine + gemcitabine vs carboplatin + gemcitabine	Gemcitabine + cisplatin 3-week vs 4-week schedule
<b>Primary endpoint</b>	mOS	Safety MTD RPTD	mPFS	ORR (pT0N0, < pT2N0)
<b>Secondary endpoints</b>	mPFS ORR Prognostic clinical markers	ORR mOS	ORR DCR mOS Toxicity HRQoL	Relapse rate Survival parameters Toxicity Dose intensity
<b>Assessment of response</b>	CT or MRI RECIST v 1.1	CT RECIST v 1.1	CT or MRI RECIST v 1.1	Pathological stage at cystectomy
<b>Assessment of toxicity</b>	NCI-CTCAE v 4.0	NCI-CTCAE v 4.0	NCI-CTCAE v 4.0	NCI-CTCAE v 4.0
<b>Assessment of HRQoL</b>	-	-	EORTC QLQ-C30 v 3.0	-
<b>Statistical analyses</b>	Descriptive statistics Pearson $\chi^2$ test Log-rank (Mantel-Cox) Kaplan Meier	Descriptive statistics Log-rank (Mantel-Cox) Kaplan Meier	Descriptive statistics Fisher's exact test Log-rank (Mantel-Cox) Cox proportional hazard Kaplan Meier Linear regression models Walds test	Descriptive statistics Pearson $\chi^2$ test Univariate regression analysis Multivariate logistic regression Flexible parametric model Kaplan Meier Relative survival model by Ederer II

RWD, real-world data; mUC, locally unresectable or metastatic urothelial cancer; MIBC, muscle-invasive bladder cancer; mOS, overall survival; MTD, maximum tolerated dose; RPTD, recommended phase II dose; mPFS, median progression-free survival; ORR, overall response rate; DCR, disease control rate; HRQoL, health-related quality of life; CT, computer tomography; MRI, magnetic resonance imaging; RECIST, Response Evaluation Criteria in Solid Tumours; NCI-CTCAE v 4.0, National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0; EORTC QLQ-C30, European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30.

### 3.1 PATIENT COHORTS

The patient cohorts in the four papers span over different stages of UC ranging from MIBC to first- and second-line treatment of mUC. The cohorts in **Papers I–III** comprised patients with non-curable unresectable (cT4b), locally advanced (N1–3), or metastatic (M1) urothelial carcinoma originating in the renal pelvic, ureter, urinary bladder, or urethra, defined as mUC. The patients in **Papers I and II** had progressed on first-line platinum chemotherapy and were treated with second-line chemotherapy. The population in **Paper III** consisted of patients treated with first-line chemotherapy who were ineligible for cisplatin due to renal impairment. We chose to exclude patients with ECOG PS  $\geq 2$ , because tolerance for combination chemotherapy is known to be low in patients with both poor PS and impaired renal function [65].

Patients with curable MIBC (cT2–cT4aN0M0) were included in **Paper IV**. Patients with cT4b and/or suspected clinical lymph node metastases were excluded, because such patients are treated with an induction therapy approach that involves more extensive chemotherapy, and also involve different treatment outcome and prognosis [116].

The patients included in the four investigations were treated at oncological centres located in Sweden, Denmark, and Finland that are affiliated with the Nordic Urothelial Cancer Oncology Group (NUCOG). The participating centres in each study are listed in Appendix A.

### 3.2 RETROSPECTIVE REAL-WORLD DATA STUDIES (PAPERS I + IV)

In **Paper I**, the first 100 second-line mUC patients treated with vinflunine after the introduction of the drug in 2009 were analysed at three Nordic cancer centres affiliated with NUCOG. The patients were treated according to routine clinical practice until progression, unacceptable toxicity, or patient's/physician's decision to stop treatment. Data were collected from patient charts as stipulated in a prespecified questionnaire covering patient and disease characteristics, and prior treatment, vinflunine treatment, toxicity, and response and survival parameters (see Appendix B). The primary endpoint was overall survival defined as time from start of vinflunine treatment to all-cause death or date of last follow-up. Secondary endpoints included mPFS, ORR, DCR and toxicity and treatment patterns. Further analyses tested previously established clinical prognostic factors (ECOG PS, Hb, presence of visceral metastases and liver metastases), as well as potentially new clinical factors for mUC.

The study reported in **Paper IV** included patients treated with two different schedules of gemcitabine and cisplatin (GC) as neoadjuvant chemotherapy prior to cystectomy in MIBC at two centres in Sweden and five in Denmark. Both these countries apply similar guidelines for neoadjuvant chemotherapy, recommending treatment for patients with stage cT2–T4bN0M0, ECOG PS 0–1, GFR  $\geq 50$  ml/min, biological age  $\leq 75$  years, and no comorbidity contradicting chemotherapy or radical cystectomy. The patients were treated according to routine clinical praxis: in Denmark with four cycles of a 3-week schedule (GC-3w: cisplatin 70 mg/m<sup>2</sup> day 1, gemcitabine 1000 mg/m<sup>2</sup> days 1 and 8, q 21 days), and in Sweden with three cycles of a 4-week schedule (GC-4w: cisplatin at 70 mg/m<sup>2</sup> day 1, gemcitabine 1000 mg/m<sup>2</sup> days 1, 8 and 15, q 28

days). Information was collected from patient charts according to a prespecified questionnaire covering patient and disease characteristics, treatment patterns, side effects, and outcome measurements (see Appendix C). The primary endpoint was pathological complete response (pT0N0) and partial response ( $pT2N0 = pT0N0$ ,  $pTisN0$ ,  $pTaN0$ , or  $pT1N0$ ) at cystectomy. Secondary endpoints were relapse rate, survival parameters (from start of neoadjuvant chemotherapy to all-cause death, bladder-cancer-specific death, or date of relapse), toxicity, and treatment patterns.

### **3.3 PROSPECTIVE CLINICAL TRIALS**

#### **3.1.1 VINSOR – a dose-finding phase I trial (Paper II)**

The addition of sorafenib to vinflunine as second-line treatment in patients with mUC was investigated in the prospective clinical dose-finding phase I trial entitled VINSOR (ClinicalTrials.gov NCT01844947). Patients with disease relapse or progression no later than 6 months after completion of previous platinum-combination treatment and with ECOG PS  $\leq 1$  were eligible for inclusion. Patients with brain metastases, congestive heart failure, angina pectoris, poorly controlled hypertension, hypercalcaemia, hypokalaemia, prolonged QTc time, impaired bone marrow or liver function, or GFR  $< 40$  ml/min at baseline were excluded. Three NUCOG centres participated in the trial.

The study was performed according to a conventional 3 + 3 dose-escalation design with the primary endpoint to define the recommended phase II dose (RPTD) by evaluating toxicity [117]. Cohorts of three patients were added at each dose step and escalated to the next dose level depending on whether a dose-limiting toxicity (DLT) did or did not occur. The dose escalation continued until at least two patients in a cohort of three to six patients experienced DLTs (i.e., maximum tolerated dose [MTD]). The RPTD was defined as the dose of sorafenib and vinflunine one dose step below the MTD. A commonly used DLT definition was applied: grade  $\geq 4$  neutropenia (absolute neutrophil count  $< 0.5 \times 10^9$  for  $\geq 7$  days or  $< 0.1 \times 10^9$  for  $\geq 3$  days); febrile neutropenia of grade  $\geq 3$  (absolute neutrophil count  $< 1.0 \times 10^9$  and temperature  $\geq 38.5$  °C); thrombocytopenia of grade  $\geq 4$  or with bleeding or need of platelet transfusion; liver toxicity (ALAT/ASAT) grade  $\geq 3$  for  $> 7$  days or any other major organ toxicity of grade  $\geq 3$ , if related to the study drugs and occurring during the two first treatment cycles.

A fixed dose of vinflunine was administrated to all patients on day 1, q21 days, at either of the following levels: 320 mg/m<sup>2</sup> if ECOG PS 0, age  $< 75$  years, no previous radiation to the pelvic region, or GFR  $> 60$  ml/min; or 280 mg/m<sup>2</sup> if ECOG PS 1, age 75–80 years, previous radiation to the pelvic region, or GFR 40–60 ml/min. In addition, patients were prescribed daily sorafenib at a dose level of 400, 600, or 800 mg on days 2–21, escalating as described above. The patients were evaluated for toxicity weekly during cycle one and thereafter every third week. Treatment was continued until progression or unacceptable toxicity, or at the patient's preference. The secondary endpoints included AEs from all treatment cycles, ORR, mPFS, and mOS. The trial

was performed according to the guidelines of Good Clinical Practice (GCP). The patient data were handled using the electronic Case Report Form (eCRF) system PheedIt.

### **3.3.2 VINGEM – a randomised controlled phase II trial (Paper III)**

The randomised controlled clinical phase II trial designated VINGEM (ClinicalTrials.gov NCT02665039) compared vinflunine and gemcitabine (VG) with gemcitabine and carboplatin (GCa) as first-line treatment in cisplatin-ineligible patients with mUC. Patients with impaired renal function (GFR 30–60 ml/min), ECOG PS  $\leq$  1, and no prior chemotherapy for metastatic disease were included. Patients with impaired liver or bone marrow function, other malignancies, and any history of serious or concurrent illness or uncontrolled medical condition were excluded. Eleven centres associated with NUCOG participated in the trial. The patients were randomised 1:1 to the experimental arm VG (vinflunine 250 mg/m<sup>2</sup> [age > 80 years and/or GFR 30–40 ml/min] or 280 mg/m<sup>2</sup> [GFR 41–60 ml/min] on day 1 and gemcitabine 1000 mg/m<sup>2</sup> on days 1 and 8, q21 days) or to the control arm GCa (carboplatin area under the curve 4.5 given on day 1 and gemcitabine 1000 mg/m<sup>2</sup> on days 1 and 8, q21 days). Treatment continued until unacceptable toxicity, progression, or patient's choice to discontinue. Primary endpoint was mPFS, defined as time from randomisation to progression or death. Secondary endpoints were ORR, DCR, mOS, toxicity, and HRQoL.

The VINGEM-trial was designed as a phase II screening trial [118] with mPFS as the primary endpoint, which implies that any eventual positive findings of the primary endpoint should be interpreted as a signal of effect. A definitive phase III trial would be necessary to confirm possible positive results. The trial was performed according to the guidelines of GCP. The patient data were handled using the eCRF system PheedIt.

## **3.4 ADVERSE EVENTS AND HEALTH-RELATED QUALITY OF LIFE**

AEs were assessed with the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI-CTCAE v 4.0) [119]. For the retrospective studies (**Papers I and IV**), only grades 3 and 4 toxicity were reported. In the prospective clinical phase I and II trials (**Papers II and III**), all toxicity grades were reported using the eCRFs. Serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) in the clinical trials were reported to the sponsor according to the study protocols. Safety reports were submitted annually to the regulatory authorities and ethics committees in the participating countries according to applicable laws and regulations.

In **Paper III**, HRQoL was measured at baseline and after every two treatment cycles, using EORTC QLQ-C30 [115] (see Appendix D). This questionnaire is composed of functional scales (global health status, physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning) and symptom scales/items (fatigue, nausea and vomiting, pain, dyspnoea, insomnia, appetite loss, constipation, and diarrhoea).



### 3.5 ETHICAL AND MEDICAL APPROVALS

Ethical approvals were obtained from the independent ethics committees in each of the participating countries for each study. Ethical and medical approvals are summarised in Appendix E. The prospective clinical trial VINSOR (**Paper II**) was approved by the Swedish and Danish medicine agencies and registered at ClinicalTrials.gov (no. NCT01844947) and EudraCT (no. 2011-004289-14). The prospective clinical trial VINGEM (**Paper III**) was approved initially by the Voluntary Harmonisation Procedures (VHP) at European Medicines Agency (EMA) and subsequently by the national medicine agencies in Sweden, Denmark, and Finland. The VINGEM trial was registered at ClinicalTrials.gov (no. NCT02665039) and EudraCT (no. 2013-002417-35), and further reported in accordance with the CONSORT guidelines for randomised controlled trials [120].

Patients in the prospective clinical trials (**Papers II and III**) were informed orally and in writing, and they provided written informed consent. The study participation was compliant with the Declaration of Helsinki [121], and the trials were performed in accordance with GCP, as well as with local laws and regulations.

### 3.6 STATISTICAL ANALYSES

Descriptive statistics was used to outline baseline characteristics, treatment patterns and safety. Differences in nominal data were assessed by the Pearson  $\chi^2$ -test and by Fisher's exact test. *P*-values  $< 0.05$  were considered statically significant, except in HRQoL-analyses in which  $< 0.01$  was used. IBM-SPSS statistics software for Windows (version 26; IBM, SPSS, Armonk, NY, USA) was used for most calculations in **Papers I, II, and IV**. In **Paper III** and for the survival analyses in **Paper IV**, the calculations were conducted using STATA software, version 15 (StataCorp LLC, College Station, TX, USA).

#### 3.6.1 Sample sizes

The power calculation in **Paper III** showed that the study had to generate 110 PFS events to be able to detect a 50% increase in mPFS between the experimental and the control arm from 5 to 7.5 months (hazard ratio [HR] of 0.67) with a one-sided significance level of 10% and a power of 80% (phase II screening trial [118]). Therefore, we decided to aim for a total of 120 patients. Due to a slow accrual rate, an amendment was approved in April 2016, allowing the number of patients needed to be decreased to 60 based on a re-calculated power sampling (an increase in 80% in mPFS from 5 to 9 months [HR 0.56],  $\alpha = 10\%$  and  $\beta = 20\%$ , generating 56 PFS events). Randomisation was based on a permuted block technique and stratified for ECOG PS 0 versus ECOG PS 1 and for presence of visceral metastases versus no detected visceral metastases.

For the dose-finding phase I trial outlined in **Paper II**, the sample size was determined depending on number of patients with DLT at each dose step, according to the protocol. A maximum of six patients were accrued at each dose level, and hence the maximum total accrual was 36 patients (18 patients for each vinflunine dose group). For the retrospective RWD studies

in **Papers I** and **IV**, all patients treated at the participating sites within a prespecified time period were included.

### 3.6.2 Analyses of nominal data

Nominal data were assessed using the Pearson  $\chi^2$ -test (**Papers I** and **II**) and Fisher's exact test (**Paper III**), i.e., response measurements (ORR, DCR, complete response [CR], partial response [PR], stable disease [SD], and progressive disease [PD]), toxicity, and treatment patterns. In **Paper IV**, univariate regression analysis was used for the nominal primary endpoints (complete and partial pathological response), and for the secondary endpoints of treatment patterns and toxicity. Outcome was reported as odds ratio (OR) with 95% confidence interval (95% CI). Further, in **Paper IV**, multivariate logistic regression analyses were performed to adjust for baseline characteristic variables (calendar period, age, sex, ECOG, GFR, and clinical T stage), reported as adjusted OR (aOR) with 95% CI.

### 3.6.3 Survival calculations

In **Papers I, II**, and **III**, univariate analyses of time to event data (PFS and OS) were performed using the log-rank (Mantel-Cox) model. In **Paper III**, HRs were calculated using the Cox proportional hazard model, and efficacy (response and survival parameters) was evaluated according to the ITT principle including all participating patients and in the per-protocol population (i.e., excluding patients that completed less than one treatment cycle). To compare the relapse-free survival, bladder-cancer-specific survival, and OS in **Paper IV**, flexible parametric models were used to estimate the HR with 95% CI [122]. Adjusted hazard ratio (aHR) models included calendar period, age, sex, ECOG, GFR, and clinical T stage.

In all four studies, the Kaplan-Meier method was used to illustrate the effect of treatment on survival and to estimate time-related measurements (including median and 5-year survival). However, in **Paper IV**, the imbalance in baseline characteristics led to heavily confounded Kaplan-Meier estimates. By using flexible parametric models that include the baseline parameters (calendar period, age, sex, ECOG, GFR, and clinical T stage), standardised survival curves were fitted, allowing the effect of treatment to vary over follow-up [122].

In **Paper IV**, we analysed survival more extensively by using the relative survival model. Relative survival measures the excess death due to bladder cancer (directly or indirectly due to the cancer diagnosis) in Sweden and Denmark, taking into account the different expected all-cause mortality in the populations in the two countries, respectively [123]. Relative survival ratio was defined as the observed survival in the study cohort divided by the expected survival of a comparable group from the general population. Expected survival in the Danish and Swedish populations were estimated using the Ederer II method from Swedish and Danish population life tables, matched by age, sex, and year of chemotherapy start. The 5-year relative survival was defined as the ratio of the observed (patient) to the expected (population) survival using a cohort approach [124]. Crude and adjusted HR within 3 years from chemotherapy start were estimated using flexible parametric models [122].

#### **3.6.4 Health-related quality of life analyses**

In **Paper III**, the mean differences in HRQoL between the two treatment groups following two cycles of treatment, after controlling for baseline values, were analysed using linear regression models and scored with 99% CI using Walds test at a significance level of 0.01. The items in the questionnaire were linearly transformed to functioning or symptom scales ranging from 0 to 100 according to the scoring manual [125]. Differences in scores were considered as small (S) if 5–9 points and as moderate (M) if 10–19 points.

## 4 RESULTS

The results of the present studies, including the most important tables and figures, are provided in detail in **Papers I–IV**. The aim of this chapter is to give an overview of the main results, and additional complementary tables and figures are included here to further clarify the data from the studies or to highlight certain interesting findings.

### 4.1 VINFLUNINE AS SECOND-LINE TREATMENT IN mUC (PAPER I)

The RWD assessment reported in **Paper I** was performed to investigate the first 100 patients treated with the novel vinca alkaloid vinflunine. In summary, the mOS in our study was similar to data reported in the landmark randomised phase III trial comparing vinflunine with BSC [4]. The results of our study confirm that vinflunine is active as second-line treatment in an unselected routine clinical cohort of patients with mUC. However, there were large inter-individual differences in survival, ranging from 0.3 to 39.7 months. The ORR was 23%, and the DCR was 55%, and these values are higher than the results noted for vinflunine in the phase III trial.

Patients treated outside well-defined clinical trials constitute a more heterogeneous cohort with regard to baseline characteristics and comorbidities. Twenty percent of the patients in **Paper I** presented with ECOG PS 2 at baseline, whereas only patients with ECOG PS 0–1 were included in the phase III vinflunine trial [4]. Patients with ECOG PS 2 showed no CRs or PRs, 20% had SD, and 80% had PD. Furthermore, patients with ECOG PS 2 had shorter mOS (4.1 vs 7.0 months,  $P = 0.001$ ) and a higher frequency of grade 3/4 toxicity (95% vs 71%,  $P = 0.026$ ) compared to patients with ECOG PS 0–1. Thus, based on our RWD, the clinical benefit of treatment with vinflunine in second-line for patients with ECOG PS  $\geq 2$  is questionable and cannot be recommended.

We further explored clinical parameters that might be of prognostic value for survival in mUC. ECOG PS  $\geq 2$  and presence of visceral metastases were found to be prognostic adverse parameters, but we could not find any significant difference regarding Hb or liver metastases in our cohort of RWD patients. Patients with ECOG PS 0–1 and no visceral metastases had long mOS (18.1 months) compared to patients with one or two risk factors (ECOG PS  $\geq 2$  and/or visceral metastases).

Additional clinical parameters with potential prognostic correlation were also investigated and are summarised in Table 2. Response (CR or PR) to vinflunine treatment was a favourable factor for survival, and the same was noted for receiving six or more cycles of platinum chemotherapy prior to the start of vinflunine treatment. No other tested parameters showed significant correlation with survival.

<b>Table 2.</b>		
<b>Clinical parameters and survival (Paper I)</b>		
<b>Parameters</b>	<b>mOS (months)</b>	<b>P-value</b>
ECOG PS 0–1 vs	7.0	0.001
ECOG PS 2	4.1	
No visceral metastases	10.6	0.008
Visceral metastases	6.0	
No liver metastases	4.4	0.386
Liver metastases	6.8	
Hb ≥ 10 g/dl	7.0	0.068
Hb < 10 g/dl	6.0	
Response (CR + PR)	13.9	< 0.001
No response (SD + PD)	6.0	
≥ 6 cycles of platinum prior to vinflunine	6.9	0.016
< 6 cycles of platinum prior to vinflunine	4.7	
< 65 years old	6.2	0.569
≥ 65 years old	6.9	
Start dose vinflunine 320 mg/m <sup>2</sup>	6.5	0.060
Start dose vinflunine 250 or 280 mg/m <sup>2</sup>	6.0	
No grade 3/4 AE	7.0	0.396
Grade 3/4 AE	6.2	
Alkaline phosphatase normal	6.8	0.058
Alkaline phosphatase elevated	4.9	
Statistically significant differences are highlighted ( $P < 0.05$ ).		
Above the dotted line are previously suggested prognostic factors for first- and second-line treatment, and below the line are other parameters tested for potential prognostic correlation.		
mOS, median overall survival; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, haemoglobin; CR, complete response; PR; partial response; SD, stable disease; PD, progressive disease; AE, adverse event.		

Grade 3/4 toxicity was high, with 76% of the patients experiencing at least one grade 3/4 AE. The most common grade 3/4 AEs were fatigue (36%), anaemia (33%), neutropenia (23%), and constipation (22%). Patients with ECOG PS 2, as compared to those with ECOG PS 0–1, had a significantly higher degree of at least one grade 3/4 AE (95% vs 71%,  $P = 0.026$ ) and fatigue (60 vs 30%,  $P = 0.012$ ). There was no correlation between the presence or absence of at least one grade 3/4 AE and survival (Table 2).

## 4.2 VINFLUNINE AND SORAFENIB AS SECOND-LINE TREATMENT IN mUC (PAPER II)

VINSOR was a conventional 3+3 dose-escalation phase I trial, which was performed to examine the novel combination of vinflunine and sorafenib as second-line treatment in mUC. Twenty-two patients were included between April 2012 and September 2017. Nineteen patients were evaluable for toxicity according to the definition of DLT (see section 3.3.1). With vinflunine at 320 mg/m<sup>2</sup>, three DLTs occurred among the first five patients given sorafenib at the lowest dose level of 400 mg, and this combination was accordingly deemed too toxic for further evaluation (Figure 4). Treatment with vinflunine 280 mg/m<sup>2</sup> reached the highest planned dose level together with sorafenib 800 mg, but three out of five patients experienced a DLT at that level-dose. Hence the MTD was determined to be the dose step below, that is, vinflunine 280 mg/m<sup>2</sup> and sorafenib 600 mg, and therefore the RPTD was set at vinflunine 280 mg/m<sup>2</sup> in combination with sorafenib 400 mg.

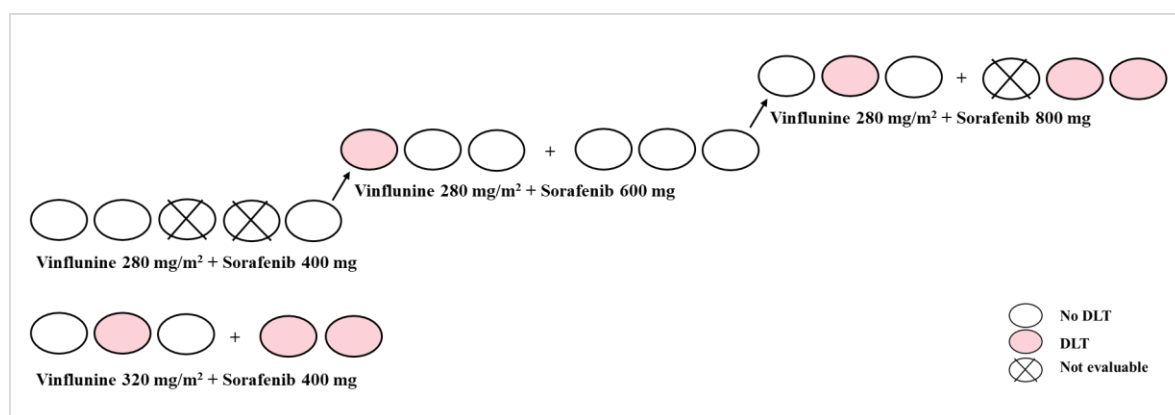


Figure 4. Schematic overview of the dose escalation in the VINSOR trial (**Paper II**). Three patients were not evaluable as they received less than two cycles of treatment and did not experience any DLT.

Toxicity was carefully monitored throughout the VINSOR trial. Overall, the toxicity profile concurred with previously described side effects for the two drugs given as monotherapy. All SAEs and AEs grade 1–5 are reported in **Paper II**. The most frequent AEs were fatigue (80%), constipation (60%), hypertension (47%), nausea (43%), and diarrhoea (42%). The most common treatment-related grade 3/4 AEs were neutropenia (32%), febrile neutropenia (26%), hypertension (16%), and hyponatraemia (16%). These AEs were most likely related to vinflunine [83], with the exception of hypertension, which has previously been described as a side effect of sorafenib [89]. The degree of hyponatraemia was higher than previously reported for vinflunine or sorafenib in monotherapy. The DLTs were febrile neutropenia ( $n = 5$ ), neutropenia ( $n = 1$ ), and hypertension ( $n = 1$ ).

Secondary endpoints in the VINSOR trial were ORR, mPFS, and mOS. Seventeen patients were evaluable for efficacy and survival. ORR was 41% (7 out of 17 patients), in all cases representing partial responses, and DCR (PR + SD) was 71% (12 out of 17 patients). Including all patients, the mPFS was 4.5 months (1.2–16.1 months), and mOS was 7.0 months (1.8–41.7 months).

### 4.3 VINFLUNINE AND GEMCITABINE AS FIRST LINE-TREATMENT IN mUC (**Paper III**)

The randomised phase II trial VINGEM (**Paper III**) compared the experimental combination of vinflunine and gemcitabine (VG) with the standard carboplatin and gemcitabine (GCa) as first-line treatment in mUC patients ineligible for cisplatin. Sixty-two patients were included between April 2014 and February 2018 and were randomised to receive VG ( $n = 32$ ) or GCa ( $n = 30$ ). Assessments were conducted according to the ITT principle ( $n = 62$ ) and also per-protocol analysis ( $n = 59$ ). Three patients in the VG arm did not receive any treatment specified in the protocol and hence were excluded from the per-protocol analysis.

In the ITT analysis, the experimental combination VG did not improve the primary endpoint mPFS compared to standard treatment with GCa (median 6.2 vs 6.3 months [HR 0.75; 95% CI 0.44–1.28;  $P = 0.293$ ]). Furthermore, there was no significant difference in mOS between the two treatments (12.5 vs 10.6 months [ $P = 0.81$ ]). Further, in the per-protocol analysis, there

was no significant difference in either mPFS or mOS between the two arms. Interestingly, ORR in the experimental arm VG was 63%, including 22% CR, and this level was significantly higher than in the GCa arm in the per-protocol analysis. Table 3 highlights the response data from Table 2 in **Paper III**.

**Table 3.**  
**Response rates in the intention-to-treat and per-protocol populations (Paper III)**

	Intention-to-treat			Per-protocol <sup>a</sup>		
	Vinflunine/ gemcitabine <i>n</i> = 32	Carboplatin/ gemcitabine <i>n</i> = 30	<i>P</i> -value	Vinflunine/ gemcitabine <i>n</i> = 29	Carboplatin/ gemcitabine <i>n</i> = 30	<i>P</i> -value
Response						
Complete response	7 (22)	1 (3)	0.054	7 (24)	1 (3)	0.026
Partial response	13 <sup>b</sup> (41)	11 (37)	0.799	13 <sup>b</sup> (45)	11 (37)	0.601
Stable disease	3 (9)	12 (40)	0.007	2 (7)	12 (40)	0.005
Progressive disease	5 (16)	6 (20)	0.746	5 (17)	6 (20)	1.000
Not evaluable	4 (13)	0	0.114	2 (7)	0	0.237
Overall response rate	20 <sup>b</sup> (63)	12 (40)	0.126	20 <sup>b</sup> (69)	12 (40)	0.037
Disease control rate	23 <sup>b</sup> (72)	24 (80)	0.558	22 <sup>b</sup> (76)	24 (80)	0.761

Data are n (%). Statistically significant differences in response rates are highlighted ( $P < 0.05$ ).

<sup>a</sup>Two patients were excluded due to adverse events before onset of treatment, and one patient was withdrawn due to infusion site reaction at first cycle.

<sup>b</sup>Confirmed responses were not available for two patients with partial response.

Overall, the side effects were manageable for treatment with VG and GCa. However, AEs were more frequently noted in the VG arm than in the control arm. The haematological toxicities, fatigue, gastrointestinal disorders, and nausea/vomiting were the most common treatment-related AEs of all grades. The most common grade 3/4 AEs were neutropenia, thrombocytopenia, anaemia, febrile neutropenia, and infection. The VG arm displayed a high grade of grade 3/4 neutropenia (62% vs 43% in the GCa arm [ $P = 0.150$ ]) and febrile neutropenia (31% vs 7% [ $P = 0.016$ ]). One patient in the VG arm died due to febrile neutropenia (grade 5 AE).

There were no statistically significant detectable differences in HRQoL between patients given VG and those receiving GCa (measured after two treatment cycles, i.e., at 7 weeks). However, moderate and small clinical differences favouring the GCa arm were found in several of the functional and symptom scales/items, see Figure 5.

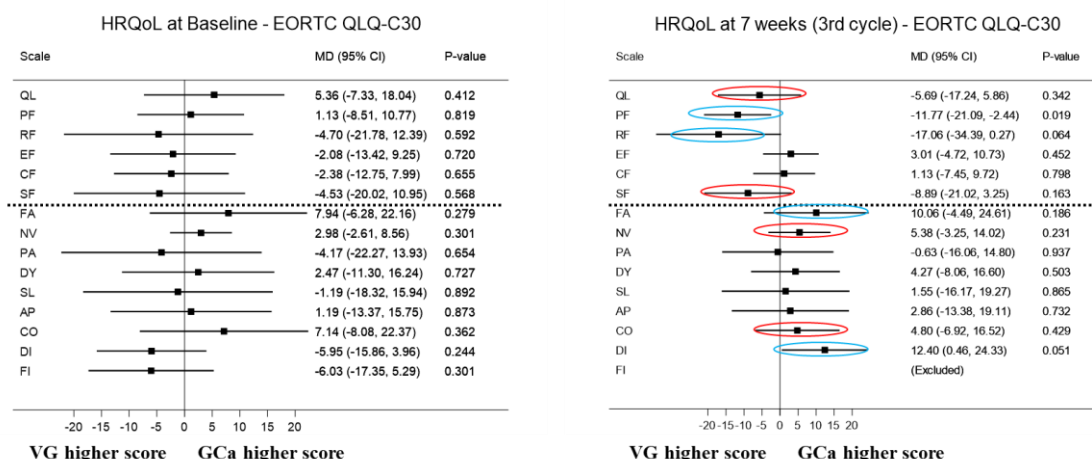


Figure 5. HRQoL at baseline and at 7 weeks (after two cycles) measured with EORTC QLQ-C30. Significance level at  $P \leq 0.01$ . MD, mean difference (VG–GCa), baseline adjusted estimates. Differences in scores are considered to be small (S) if 5–9 points (marked in red) and as moderate (M) if 10–19 points (marked in blue). The *functional scales* (QL, global health status; PF, physical functioning; RF, role functioning; EF, emotional functioning; CF, cognitive functioning; SF, social functioning) are presented above the dotted line, and the *symptom scales/items* (FA, fatigue; NV, nausea and vomiting; PA, pain; DY, dyspnoea; SL, insomnia; AP, appetite loss; CO, constipation, DI, diarrhoea; FI, financial item) are shown below the dotted line.

#### 4.4 GEMCITABINE AND CISPLATIN IN MIBC (PAPER IV)

**Paper IV** is a retrospective study of MIBC patients treated with neoadjuvant chemotherapy, which compared two separate GC schedules with different dose intensities and total doses of cisplatin and gemcitabine, and evaluated implications for efficacy and safety. Data were collected from a total of 706 patients, 251 treated with GC-4w at two centres in Stockholm, Sweden, and 455 treated with GC-3w at five centres in Denmark. The patients were treated between January 2010 and June 2018 in Sweden, and between January 2013 and June 2018 in Denmark. Adjustments for calendar period, age, sex, ECOG PS, GFR, and clinical T stage were made in the analyses of efficacy and safety due to imbalances in baseline characteristics.

For the primary endpoint, the more cisplatin-dose-intense GC-3w schedule showed a significantly higher pathological response (pT0N0 and < pT2N0) compared to the GC-4w schedule. Complete response was achieved in 46% of the GC-3w patients compared to 32% of the GC-4w subjects (OR 1.85; 95% CI 1.33–2.57;  $P < 0.005$ ). Corresponding values for downstaging to non-muscle-invasive disease were 60% and 47%, respectively (OR 1.67; 95% CI 1.21–2.29;  $P < 0.005$ ). After adjusting for the imbalance in baseline characteristics, the significant difference in pT0N0 between the two schedules was still valid, but not for downstaging to < pT2N0. There were indications of an association with higher rates of pathological response for patients with GFR  $\geq 70$  and lower clinical stage (Table 4).



	pT0N0	< pT2N0	All-cause survival	Bladder-cancer-specific survival	Relapse-free survival
	OR (95% CI) <sup>a</sup>	OR (95% CI) <sup>a</sup>	HR (95% CI) <sup>b</sup>	HR (95% CI) <sup>b</sup>	HR (95% CI) <sup>b</sup>
Chemotherapy					
GC-4w	1	1	1	1	1
GC-3w	1.74 (1.12–2.71)	1.05 (0.68–1.62)	1.32 (0.87–2.01)	1.26 (0.81–1.97)	1.25 (0.85–1.84)
Calendar period					
2010–2012	1	1	1	1	1
2013–2015	0.83 (0.47–1.48)	1.32 (0.75–2.32)	0.86 (0.52–1.40)	0.85 (0.50–1.43)	0.68 (0.44–1.07)
2016–2018	0.54 (0.29–0.98)	0.93 (0.52–1.68)	0.78 (0.46–1.33)	0.80 (0.45–1.40)	0.70 (0.44–1.13)
Age interval					
34–59 years	1	1	1	1	1
60–69 years	1.04 (0.70–1.56)	1.03 (0.69–1.54)	0.99 (0.68–1.45)	0.93 (0.62–1.38)	0.80 (0.57–1.14)
70–80 years	1.41 (0.90–2.22)	1.43 (0.91–2.26)	0.88 (0.57–1.36)	0.77 (0.48–1.23)	0.71 (0.47–1.05)
Sex					
Male	1	1	1	1	1
Female	0.92 (0.64–1.31)	0.71 (0.50–1.02)	1.62 (1.18–2.22)	1.73 (1.24–2.42)	1.73 (1.29–2.32)
ECOG PS					
0	1	1	1	1	1
1	0.71 (0.43–1.20)	0.60 (0.36–0.99)	1.22 (0.78–1.91)	1.21 (0.75–1.95)	1.26 (0.84–1.89)
Missing data	0.90 (0.45–1.81)	1.34 (0.63–2.81)	1.13 (0.60–2.13)	0.83 (0.38–1.80)	0.83 (0.42–1.66)
GFR					
< 70 ml/min	1	1	1	1	1
≥ 70 ml/min	1.93 (1.16–3.20)	1.87 (1.16–3.01)	0.79 (0.52–1.18)	0.81 (0.52–1.25)	0.68 (0.47–0.99)
cTNM					
cT2N0	1	1	1	1	1
cT3N0	0.67 (0.43–1.03)	0.45 (0.29–0.69)	1.46 (1.00–2.16)	1.60 (1.06–2.43)	1.34 (0.93–1.91)
cT4N0	0.32 (0.13–0.83)	0.26 (0.11–0.59)	2.22 (1.23–3.99)	2.51 (1.35–4.64)	2.03 (1.18–3.51)
cTxN0	0.91 (0.60–1.36)	1.10 (0.72–1.67)	0.88 (0.58–1.34)	1.04 (0.67–1.63)	0.90 (0.60–1.34)

Statistically significant differences are highlighted.

<sup>a</sup>Multivariate logistic regression model.

<sup>b</sup>Multivariate flexible parametric model.

OR, odds ratio; CI, confidence interval; HR, hazard ratio; GC-4w, gemcitabine and cisplatin 4-week schedule; GC-3w, gemcitabine and cisplatin 3-week schedule; ECOG, Eastern Cooperative Oncology Group; GFR, glomerular filtration rate.

Interestingly, despite the higher rate of pathological response with the three-week schedule, we did not find any overall, bladder-cancer-specific, or relapse-free survival advantage in patients treated with GC-3w compared to those given GC-4w. The Kaplan-Meier survival curves were adjusted for the imbalance in baseline characteristics, again showing no significant differences in survival. We further analysed excess death by considering differences in the background mortality in Sweden and Denmark. This sensitivity analysis did not either show any significant differences in survival between the two treatment groups, and it yielded survival curves that were similar to the Kaplan-Meier curves for bladder-cancer-specific survival. This contradictory finding, that the significantly higher proportion of complete responses did not translate into increased survival for patients treated with the GC-3w schedule, is given further consideration in the Discussion (Chapter 5). In the multivariate flexible parametric model, female sex and higher clinical stage were associated with poorer survival (Table 4).

Figure 6 illustrates the hazards for bladder-cancer relapse, all-cause death, and bladder-cancer-specific deaths. A notable observation is the high risk of relapse within the first two years after neoadjuvant chemotherapy, which translates into an increased risk of death within the first years after treatment. The median follow-up was shorter in the GC-3w group than in the GC-4w patients (2.7 vs 3.6 years), and thus the hazard curves should be interpreted with caution after the third year. Furthermore, the time from relapse to death was unexpectedly short (6.2

months with the GC-4w schedule and 5.1 months with GC-3w), indicating a highly aggressive disease following relapse after initial cisplatin-based combination chemotherapy.

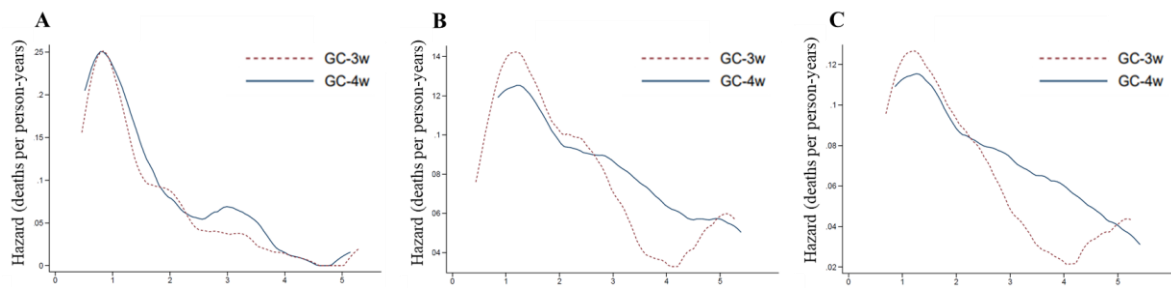


Figure 6. Hazard curves for bladder-cancer relapse (A), all-cause death (B), and bladder-cancer death (C) for GC-4w versus GC-3w

Side effects were manageable for both treatment schedules. A significantly higher degree of grade 3/4 neutropenia was seen in the GC-3w compared to GC-4w group (44% vs 36% [ $P = 0.032$ ]), although this difference was not statistically significant after adjusting for the imbalance in the baseline characteristics. Accordingly, G-CSF was used significantly more often in the GC-3w than in the GC-4w treatment (27% vs 8% [ $P < 0.005$ ]) in the unadjusted model but not in the adjusted model. No difference was observed between the two schedules with regard to frequency of febrile neutropenia. More patients with the GC-3w schedule terminated the treatment prematurely and experienced at least one dose delay, mainly due to neutropenia. Dose reduction and omitted doses were more common in the GC-4w than in the GC-3w group. Moreover, a particularly low dose intensity was seen for gemcitabine on day 15, where dose reduction occurred in at least one cycle in 30% and omitted dose in 47%, most commonly due to thrombocytopenia and neutropenia.

## 5 DISCUSSION

In this chapter, the results of the present studies are discussed in a broader context and compared with published literature. The aim is to discuss chemotherapy in MIBC and mUC from a more general perspective in comparison with the current findings. Furthermore, several methodological concerns must be considered while interpreting our observations.

### 5.1 METHODOLOGICAL CONSIDERATIONS

#### 5.1.1 Retrospective real-world data studies

Randomised clinical trials are considered to be the gold standard in clinical oncological research. Nevertheless, RWD studies have the advantage of enabling analysis of more heterogeneous populations that more accurately reflect the real-world setting regarding aspects such as PS [126], comorbidities, and age. Moreover, RWD studies can provide data on subgroups, treatment sequences, and endpoints not included in clinical trial [127, 128]. The number of approved drugs and treatment options in advanced UC is rapidly increasing, thus it is of the utmost importance to use RWD to confirm the efficacy and safety of the novel treatment options in the real-life setting, as was done in the study outlined in **Paper I**. In a broader perspective, RWD can be utilised for health care decisions and estimations of cost effectiveness of the introduction of new drugs. An example of this is that the results reported in **Paper I** have been used as a comparator for the introduction of ICIs in second-line mUC in Sweden [129].

Retrospective RWD studies are limited in terms of the accuracy of the data collection, which leads to the risk of bias from several cofounders. In both **Papers I and IV**, there were a number of parameters that could not be controlled for. In those investigations, the clinical routines differed slightly between the participating centres with respect to staging procedures, health care management, and follow-up programmes, all of which might have had an impact on the evaluated efficacy parameters. For example, in **Paper III**, no information was recorded regarding surgical outcomes for pre-staging TUR-B and cystectomy (i.e., number of lymph nodes resected, positive surgical margins, and peri-operative morbidity and mortality) and oncological treatment after relapse, all important factors that might have influenced the survival outcomes in the study. In addition, data on other significant factors such as comorbidity burden, socio-economic factors, and smoking history were not included. Further, it is important to adjust for differences in baseline characteristics when comparing two different treatment options in a non-randomised study (**Paper IV**) in order to overcome the inevitable imbalance that occurs in routine cohorts. Still, there is a risk of bias from unknown cofounders despite careful adjustments.

The assessment of toxicity in RWD studies is influenced by less frequent healthcare visits, and less accurate documentation of side effects compared to the meticulous reporting of side effects in clinical trials. Side effects of all grades may be underestimated, although grade 3 and 4 AEs

are more probably to be reported [128]. Further, efficacy data must be interpreted carefully due to lack of standardised assessments such as the use of Response Evaluation Criteria in Solid Tumours (RECIST) criteria or central assessment of pathological reports in ordinary clinical use. Nonetheless, in the study outlined in **Paper I**, the patients were subjected to a retrospective follow-up evaluation with RECIST, an approach that is not possible when studying larger cohorts.

### 5.1.2 Prospective clinical trials

Performing academic clinical trials is challenging in that it is associated with high costs and numerous regulatory formalities, and it is time consuming. In the rapidly evolving treatment landscape for mUC, the possibility to include patients in an ongoing trial can potentially change during the course of the study, as exemplified by the VINGEM trial, in which introduction of ICIs in first-line treatment made it more difficult to include patients (**Paper III**). Furthermore, there are limitations in accrual of first-line ineligible and second-line mUC patients in trials due to the rather limited number of patients with this diagnosis and the usually fragile patient populations, two aspects constituting a clear difficulty in both the VINSOR and the VINGEM trial (**Papers II and III**).

Phase I dose-escalation trials aim to minimise the number of subjects exposed to unknown toxicity and maintain rapid accrual to identify the MTD of the experimental drug or the novel drug combination. Further, phase II and III trials are necessary to evaluate the efficacy of the novel drug or drug combination. Therefore, the efficacy data that are described regarding the VINSOR trial (**Paper II**) are merely descriptive.

VINGEM (**Paper III**) was designed as a phase II screening trial with the primary endpoint mPFS [118], and thus any positive results should be interpreted as a signal of effect and must be confirmed in a definitive phase III trial. The main limitation of VINGEM was that it had to be downsized from 120 to 60 patients due to a slow accrual rate, which reduced the statistical power and the possibility to detect statistically significant differences between the two treatment arms.

## 5.2 NEOADJUVANT TREATMENT IN MIBC

Cisplatin-based neoadjuvant chemotherapy prior to cystectomy improves survival in patients with MIBC, but the optimal neoadjuvant regimen still remains unknown. No randomised clinical phase III trials comparing different regimens have yet been finally reported, although in France there is an ongoing randomised phase III trial (VESPER) comparing dd-MVAC with a 3-week schedule of GC, and it is expected that the final results of this study will be presented in mid 2021 [130]. In the study described in **Paper IV**, we found that a more cisplatin-dose-intense 3-week schedule of GC, as compared to a commonly used 4-week schedule, was associated with significantly higher rates of pathological complete response (pT0N0) and downstaging to non-muscle-invasive disease (<pT2N0). The pathological response rates for

the two schedules were in the same range or exceeded numbers reported in the literature from other neoadjuvant trials using different cisplatin-based regimens [38-41, 44, 45].

Downstaging to pT0N0 or < pT2N0 has been suggested as a surrogate marker for survival following neoadjuvant treatment in patients with MIBC [49, 50]. Notably, despite the higher rate of pathological response in patients treated with GC-3w compared to those with GC-4w in **Paper IV**, no significant differences in survival outcomes could be detected between the two schedules. This peculiar finding indicates that, in addition to downsizing the primary tumour in the bladder, an important role of neoadjuvant chemotherapy is to eradicate distant micro-metastases. Approximately one third of the patients in our study did relapse, with no significant difference between GC-3w and GC-4w, indicating possible heterogeneity in cisplatin-sensitivity between tumour cells of the primary tumour in the bladder and circulating micro-metastases. To circumvent the cisplatin resistance, it appears to be important to combine cisplatin with drugs with different mechanisms of action rather than to further increase the dose and intensity of the cisplatin-based chemotherapy.

Patients with residual tumour ( $\geq$  pT2N0 and/or node-positive disease) after neoadjuvant chemotherapy have a poor prognosis; in **Paper IV**, the 5-year survival for such patients was only 45% compared to 90% for those with pT0N0. In line with perioperative treatment in other cancer diagnoses (e.g., breast cancer [131]), both neoadjuvant and adjuvant treatment might be of value for selected patients. To address that issue, better response assessments are needed to individualise the treatment. Measuring circulating tumour DNA (ctDNA) after neoadjuvant chemotherapy and cystectomy is one possible strategy that might enable selection of patients that may benefit from additional adjuvant treatment, preferable with drugs with different mechanisms of action than platinum-compounds [132, 133].

### 5.3 FIRST-LINE TREATMENT IN mUC

Cisplatin combination chemotherapy is the treatment of choice in first-line mUC. However, approximately half of mUC patients are not eligible for cisplatin, and the ineligible population shows inferior survival compared to eligible mUC patients given cisplatin-based treatment. In the randomised phase II trial VINGEM (**Paper III**), we explored vinflunine in combination with gemcitabine in patients ineligible for cisplatin due to renal impairment. No statistical differences in mPFS or mOS were seen between VG and the control arm GCa, although the experimental arm with VG did show a notably high frequency of responses. The ORR for patients treated with VG exceeds the numbers reported from other trials with cisplatin-ineligible patients and is comparable to the best response data reported for all systemic treatments in mUC (see Table 5). The ORR of 63% makes VG a promising treatment option, but this novel combination must be further explored in larger trials with mUC patients. Also, the VG combination would be interesting to investigate as neoadjuvant treatment for MIBC patients with renal impairment, for whom no treatment options are presently available, to possibly achieve high pathological response rates at cystectomy.

<b>Table 5.</b>							
<b>Response and survival in first-line treatment</b>							
<b>Authors</b>	<b>Study treatment</b>	<b>Study design</b>	<b>ORR (%)</b>	<b>CR (%)</b>	<b>mPFS (months)</b>	<b>mOS (months)</b>	
<b>First-line cisplatin-eligible patients</b>							
Sternberg <i>et al.</i> 1989 [3]	MVAC	Single arm phase II	72	36	NR	14.8	
Sternberg <i>et al.</i> 2001, 2006 [56, 57]	HD-MVAC vs MVAC	Randomised phase III	64 vs 50	21 vs 9	9.5 vs 8.1	15.1 vs 14.9	
von der Maase <i>et al.</i> 2000, 2005 [36, 58]	GC vs MVAC	Randomised phase III	49 vs 46	12 vs 12	7.7 vs 8.3	14.0 vs 15.2	
Bellmunt <i>et al.</i> 2012 [62]	PCG vs GC	Randomised phase III	56 vs 44	14 vs 11	8.3 vs 7.6	15.8 vs 12.7	
<b>First-line cisplatin-ineligible patients</b>							<b>Criteria for “unfit”</b>
Holmsten <i>et al.</i> 2019 ( <b>Paper III</b> )	VG vs GCa	Randomised phase II	63 vs 40	22 vs 3	6.2 vs 6.3	12.5 vs 10.6	GFR 30–60
De Santis <i>et al.</i> 2016 [75]	VG vs VCa	Randomised phase II	44 vs 28	6 vs 11	5.9 vs 6.1	14.0 vs 12.8	GFR 30–60
De Santis <i>et al.</i> 2009, 2012 [65, 66]	GCa vs M-CAVI	Randomised phase III	41 vs 30	3 vs 3	5.8 vs 4.2	9.3 vs 8.1	GFR 30–60 ECOG PS 2
Balar <i>et al.</i> 2017 [67] O'Donnell <i>et al.</i> 2019 [68]	Pembrolizumab	Single arm phase II	29	9	NR	11.3	GFR 30–60 ECOG PS 2 Hearing loss Peripheral - neuropathy Heart failure
Balar <i>et al.</i> 2017 [69]	Atezolizumab	Single arm phase II	23	9	2.7	15.9	GFR 30–60 ECOG PS 2 Hearing loss Peripheral - neuropathy

<sup>a</sup>At least one of the following criteria.

The response rates in the VINGEM trial (Holmsten *et al.*) are highlighted for comparison.

ORR, overall response rate; CR, complete response; mPFS, median progression-free survival; mOS, median overall survival; MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin; HD-MVAC, high-dose MVAC; GC, gemcitabine and cisplatin; PCG, paclitaxel, cisplatin, and gemcitabine; VG, vinflunine and gemcitabine; GCa, gemcitabine and carboplatin; GFR, glomerular filtration rate, ml/min; M-CAVI, methotrexate, carboplatin, and vinblastine; ECOG PS, Eastern Cooperative Oncology Group performance status; VCa, vinflunine and carboplatin; NR, not reported.

Patients that are cisplatin-ineligible are heterogeneous, and different criteria for being unfit have been used. In the VINGEM trial (**Paper III**), impaired renal function was the only criterion applied to define cisplatin ineligibility, which constitutes a homogeneous patient cohort. Other trials have included patients with various parameters for being cisplatin ineligible (e.g., impaired renal function, ECOG PS 2, congestive heart failure, impaired hearing, and peripheral neuropathy) (Table 5), which reduces the possibility of cross-study comparison. The VG combination was also evaluated in the JASINT1 trial [75], which used the same definition for unfit as applied in the VINGEM trial, and the outcome data in these two trials were similar regarding efficacy and toxicity. Only one randomised phase III trial with cisplatin-ineligible patients (M-CAVI compared to GCa) has been performed [65, 66], and, in that investigation, patients with two criteria for being unfit for cisplatin (ECOG PS 2 and GFR 30–60 ml/min) showed a low response rate, short mOS, and a high degree of AEs. It seems that patients with both renal impairment and poor PS do not benefit from combination chemotherapy and should be considered for mono-chemotherapy, drugs with alternative mechanisms of action, inclusion in clinical trials, or BSC.

In the single-arm phase II trials for treatment with ICIs in first-line cisplatin-ineligible patients [67, 69], the majority of patients were ineligible to cisplatin due to renal impairment, but patients with ECOG PS 2, heart failure, peripheral neuropathy, and hearing loss were also

included. Two large ongoing trials including both cisplatin-eligible and ineligible patients in first-line treatment are assessing ICIs in monotherapy in comparison with ICIs combined with chemotherapy and chemotherapy alone [134, 135]. These trials will provide important information about the benefit of the available treatment options in first-line mUC. Hopefully, additional knowledge will also be obtained about the efficacy and safety profile of ICIs and carboplatin-combination chemotherapy in the different subgroups within the cisplatin-ineligible population

The toxicity profile is well known for cisplatin and carboplatin combinations and is illustrated schematically in Table 6. The side effects in the experimental arm VG in the VINGEM trial (**Paper III**) were reasonable, although a higher degree of AEs were reported for VG than for standard GCa. The high degree of neutropenia and febrile neutropenia observed in the VG arm is of special concern and was higher than reported for treatment with VG in the JASINT1 trial [75]. However, the start dose of gemcitabine was lower in the JASINT1-trial, which may partly explain the observed differences. Given the high frequency of neutropenia and associated febrile neutropenia for VG in the VINGEM trial, adjusted doses or treatment with G-CSF may be considered in future trials with VG.

**Table 6.**  
**Schematic diagram of selected adverse events grade 3/4 for first-line treatment options**

	Vinflunine/ gemcitabine <sup>a</sup>	Gemcitabine/ carboplatin <sup>b</sup>	Cisplatin combinations <sup>c</sup>	
Anaemia				≥ 55%
Neutropenia <sup>d</sup>				
Febrile neutropenia <sup>e</sup>				30–54%
Thrombocytopenia				
Infection				10–29%
Fatigue				
Nausea/vomiting				< 10%
Constipation				

<sup>a</sup>Holmsten *et al.* 2019 (**Paper III**), De Santis *et al.* 2016 [75].

<sup>b</sup>Holmsten *et al.* 2019 (**Paper III**), De Santis *et al.* 2012 [66].

<sup>c</sup>GC and MVAC, von der Maase *et al.* 2000 [36]; PCG, Bellmunt *et al.* 2012 [62].

<sup>d</sup>For vinflunine/gemcitabine, very high in Holmsten *et al.* 2019, high in de Santis *et al.* 2016.

<sup>e</sup>For vinflunine/gemcitabine, high in Holmsten *et al.* 2019, low in de Santis *et al.* 2016.

GC, gemcitabine and cisplatin; MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin; PCG, paclitaxel, cisplatin, and gemcitabine.

## 5.4 SECOND-LINE TREATMENT AND BEYOND IN mUC

At the time the present thesis was initiated, vinflunine had recently been introduced in routine clinical practice as standard second-line treatment after progression on platinum chemotherapy. As the survival advantage with vinflunine is small (2.6 months in comparison with BSC) [4], and the toxicity profile of this drug may be a concern, especially regarding constipation and neutropenia, it is clearly important to evaluate vinflunine in an unselected clinical routine cohort. In **Paper I**, we confirmed that vinflunine is an active drug in everyday clinical use, with mOS and side effects comparable to those reported in the landmark phase III trial of vinflunine [4]. Furthermore, the results in Paper I agree with other RWD studies of vinflunine that have

been published after our trial was conducted [128, 136]. Vinflunine has also been included as the control arm (investigators' choice of vinflunine, paclitaxel, or docetaxel) in the randomised phase III trials for pembrolizumab and atezolizumab in second-line treatment [94, 95]. In the atezolizumab trial, the mOS for patients treated with vinflunine was 8.3 months, which was longer than expected based on previous investigations. Efficacy data from selected studies of second-line treatment are presented in Table 7 for comparison with the findings in **Paper I**. In summary, data from both randomised trials and RWD confirm the role of vinflunine as an active option for treatment of platinum-resistant mUC patients.

**Table 7.**  
**Response and survival in second-line treatment**

Authors	Study treatment	Study design	ORR (%)	mPFS (months)	mOS (months)
Holmsten <i>et al.</i> 2016 ( <b>Paper I</b> )	Vinflunine	Retrospective RWD	23	2.8	6.3
Shah <i>et al.</i> 2019 ( <b>Paper II</b> )	Vinflunine + sorafenib	Dose-finding phase I	41	4.5	7.0
Bellmunt <i>et al.</i> 2009 [4]	Vinflunine vs BSC	Randomised phase III	9 vs 0	3.0 vs 1.5	6.9 vs 4.3
Bamias <i>et al.</i> 2019 [128]	Vinflunine	Meta-analysis retrospective RWD	19	2.3 to 6.2	5.2 to 11.9
Bellmunt <i>et al.</i> 2017 [95]	Pembrolizumab vs chemotherapy <sup>a</sup>	Randomised phase III	21 vs 11	2.1 vs 3.3	10.3 vs 7.4
Powles <i>et al.</i> 2018 [94]	Atezolizumab vs chemotherapy <sup>a</sup>	Randomised phase III	13 vs 13 <sup>b</sup>	2.1 vs 4.0 <sup>b</sup>	8.6 vs 8.0 <sup>b</sup> 8.3 for vinflunine

<sup>a</sup>Investigators' choice of vinflunine, docetaxel, or paclitaxel.

<sup>b</sup>In the intention-to-treat population.

The OS for the RWD trial of vinflunine (Holmsten *et al.*) and the ORR from the VINSOR trial (Shah *et al.*) are highlighted for comparison.

ORR, overall response rate; mPFS, median progression-free survival; mOS, median overall survival; RWD, real-world data; BSC, best supportive care.

The approval of ICIs in 2017 introduced a new approach with a principally different mechanism of action in mUC patients progressing on platinum treatment. Furthermore, the fibroblast growth factor receptor (FGFR) inhibitor erdafitinib [137] and the antibody-drug conjugate (ADC) targeting Nectin-4 enfortumab vedotin [138] were recently approved by the FDA for treatment in second line or beyond. Intense research is also ongoing to investigate chemotherapy in combination with drugs with different mechanisms of action to improve response and survival in platinum-resistant mUC patients. In the phase I trial VINSOR (**Paper II**), we show that the novel treatment combination of vinflunine and the antiangiogenic VEGFR multi-tyrosine kinase inhibitor sorafenib offers a tolerable and safe treatment. The reported ORR, mPFS, and mOS are shown in Table 8, and the observed ORR of 41% and DCR of 71% are notable and exceed the corresponding numbers noted in other second-line trials. The higher response rate might be attributed to the addition of sorafenib, or perhaps simply to chance considering the limited sample size; phase I trials are not designed to evaluate efficacy parameters. In comparison, a randomised phase II trial assessing GC plus sorafenib or GC alone in first-line treatment showed no additional response or survival effect of sorafenib [139]. Also, another VEGFR antagonist, ramucirumab, has been observed to have initial promising effects in platinum-refractory mUC patients, with improved mPFS in combination with docetaxel



compared to docetaxel alone, although this did not translate into prolonged survival in patients receiving ramucirumab [140, 141]. Treatment strategies targeting angiogenesis, including trials with vinflunine in combination with sorafenib may be further explored in selected patients.

Treating patients in second or third-line and beyond in mUC is a medical challenge, since a considerable proportion of the patients present with a large tumour burden and multiple tumour-associated symptoms. Moreover, patients in late-stage disease often present with an overall poor PS which makes them more susceptible to side effects of systemic treatments. In **Paper I**, patients with ECOG PS 2 showed less efficacy of vinflunine treatment and more side effects. Patients with ECOG PS  $\geq 2$  are generally excluded from clinical trials [126], which was also the case in the randomised phase III trials of vinflunine [4], pembrolizumab [95], and atezolizumab [94] in second-line mUC, as well as in the single-arm phase II trials with enfortumab vedotin in third-line mUC [138] and erdafitinib in second-line mUC [137]. The impact of poor performance status in treatment with ICIs remains unknown, although a recent retrospective RWD study indicated that patients with ECOG PS  $\geq 2$  show similar survival as those with ECOG PS 0–1 in second-line treatment [142]. The clinical benefit of systemic treatment in second-line or beyond for mUC patients with ECOG PS  $\geq 2$  is questionable in light of the today present knowledge, and patients should be treated with caution and preferably, if possible, within clinical trials.

## 5.5 PROGNOSTIC CLINICAL FACTORS AND MOLECULAR MARKERS

Patients with late stage mUC are highly heterogeneous regarding both clinical characteristics and sensitivity to the administered treatment drug. There is an urgent need for validated clinical and molecular prognostic and predictive markers to enable better individualisation of the treatment. For chemotherapy in mUC, prognostic clinical factors for survival are established for first- and second-line treatment [103, 105]. In **Paper I**, we evaluated the proposed prognostic parameters for mUC and confirmed that poor PS and the presence of visceral metastases were associated with shorter survival. Clinical prognostic factors for treatment of ICIs are still unknown, although a recent study suggested a five-factor model that correlates with survival, including ECOG PS  $> 1$ , presence of liver metastases, platelet count, neutrophil-lymphocyte ratio, and serum lactate dehydrogenase [143].

Extensive research is in progress regarding molecular markers associated with the efficacy of chemotherapy in UC. It is possible that the establishment of a molecular classification can aid selection of patients that are suitable for chemotherapy, as well as for ICIs and targeted therapy. For example, patients with basal differentiation appear to benefit from cisplatin-based chemotherapy and ICIs, whereas patients in the subgroup of “luminal infiltrated” do not seem to benefit from chemotherapy but from ICIs. FGFR is overexpressed in the “luminal-papillary” subgroup, and patients in that category are likely to benefit from FGFR inhibitors such as erdafitinib [22, 25]. Also, alterations in DDR genes have been associated with response to platinum-based chemotherapy in MIBC and mUC [144, 145], although further studies are

required to determine whether DDR genes can serve as a reliable molecular marker in UC [107].

The responses to ICIs are usually durable. However, only approximately 15–25% of the patients with mUC do respond to such treatment, which highlights the need for molecular markers to select patients that will benefit from immunotherapy. The expression of PD-L1 has been suggested as a predictive biomarker, but its significance in advanced UC is still unclear [24, 110]. Extensive research is in progress to identify potential molecular markers for ICIs, such as mutations in DDR genes, tumour mutational burden, and microsatellite instability, although the accuracy and robustness of possible markers must be further evaluated and tested in prospective clinical trials [24, 110, 146, 147].

## 5.6 ADVERSE EVENTS AND HEALTH-RELATED QUALITY OF LIFE

In the search for more effective drugs or treatment combinations in cancer care, side effects and HRQoL must always be taken into consideration. Higher doses of chemotherapy or more dose-intense treatment usually results in better tumour control but at the price of more toxicity, and hence it is a question of achieving a balance between efficacy of the treatment regimen and the toxicity profile. A higher degree of side effects is usually accepted for patients with MIBC given curative neoadjuvant treatment prior to radical cystectomy than for patients with mUC in the palliative setting for whom HRQoL during the remainder of life is of primary importance.

HRQoL assessment is a valuable instrument to more accurately investigate how the disease and side effects of the cancer treatment influence the patients in everyday life. Nonetheless, data are limited regarding HRQoL in relation to systemic treatment in UC [113]. The sparse knowledge available today stems primarily from patients in clinical trials that represent highly selected populations, and data on HRQoL from RWD studies are limited. Furthermore, information is usually obtained using general questionnaires such as the EORTC QLQ-C30 and not cancer-diagnosis-specific questionnaires. This was also the case for the HRQoL analyses in the VINGEM trial (**Paper III**), in which no significant differences in HRQoL were observed between the treatment arms. However, small and moderate clinical differences in favour of the control arm GCa were noted in that trial, which were probably related to the overall higher frequency of all grades of AEs reported for the experimental arm VG. After initiation of the VINGEM trial, both a diagnosis-specific questionnaire for MIBC (QLQ-BLM30) and a cross-diagnostic questionnaire for HRQoL in palliative cancer care patients (QLQ-C15-PAL) were developed [114]. Addition of these questionnaires to the basic QLQ-C30 in the VINGEM trial probably would have provided further important information that might have better reflected the HRQoL of the included patients.

For patients treated with chemotherapy for various metastatic solid tumours, it has been noted that the use of patient-reported outcomes (PROs) in addition to routine clinical visits are significantly associated with increased survival as compared to usual outpatient care alone [148]. Improving symptom management by use of PROs can also lead to better HRQoL, less

frequent admissions to the hospital, and that patients can remain on chemotherapy longer [149]. Disease-specific PRO items for mUC patients were recently suggested [150]. Still, much remains to be determined about how different treatments affect HRQoL in advanced UC [113], and future prospective clinical trials should preferably include HRQoL assessment with disease-specific questionnaires, as well as PRO strategies if possible.

## 6 CONCLUSIONS

### Paper I

- Vinflunine was confirmed to be an active drug as second-line treatment in a routine clinical cohort of patients with metastatic urothelial cancer (mUC).
- Poor performance status and the presence of visceral metastasis were negative clinical prognostic factors for vinflunine treatment.
- Patients with ECOG PS 2 showed a short overall survival and a high degree of severe toxicity.

### Paper II

- Vinflunine and sorafenib could be safely combined for treatment of second-line mUC patients, and a recommended phase II dose was identified.
- The most common dose-limiting toxicity was febrile neutropenia.
- The novel combination of vinflunine and sorafenib generated clinically meaningful disease stabilisation and tumour responses.

### Paper III

- Vinflunine and gemcitabine (VG) did not improve the progression-free survival compared to standard treatment with gemcitabine and carboplatin as first-line treatment in cisplatin-ineligible patients with mUC.
- The experimental combination of VG showed a high overall response rate including a notably high degree of complete responses.
- The VG combination was generally tolerable, albeit with a high degree of neutropenia and febrile neutropenia. No differences in health-related quality of life were observed between the two treatment arms.

### Paper IV

- Gemcitabine plus cisplatin given as a more cisplatin-dose-intense 3-week schedule compared to a commonly used 4-week schedule in muscle muscle-invasive bladder cancer resulted in significant differences in pathological response rates in favour of the 3-week schedule.
- No differences in survival were seen between the two schedules.
- The toxicity profile was manageable in both schedules, but more neutropenia and premature treatment termination occurred in patients treated with the 3-week schedule.

## 7 FUTURE PERSPECTIVES

This thesis is focused on treatment efficacy, safety and HRQoL in patients undergoing chemotherapy for advanced UC. Until recently, chemotherapy was the only option for systemic treatment of patients with advanced UC. Increased knowledge of the biology of UC and the introduction of immunotherapy have dramatically altered the clinical management of this disease over the past years. Extensive research is ongoing with several novel drugs, including ICIs, drugs targeting angiogenesis, tyrosine kinase inhibitors (e.g., FGFR inhibitors), and ADC [151]. Furthermore, numerous trials are evaluating combinations of these drugs in different stages of UC. At present, more than 200 clinical trials are ongoing that are focused on advanced UC [152]. The FGFR inhibitor erdafitinib [137] and the ADC enfortumab vedotin [138] were recently approved by the FDA for treatment in second-line or beyond, and it is expected that these drugs will soon be available for clinical use in Europe and Sweden. Even though the introduction of ICIs and targeted therapies represents a major breakthrough in the treatment of patients with mUC, the response rates and OS are still limited [67, 69, 94-96, 137, 138]. Accordingly, chemotherapy is likely to remain a cornerstone in systemic oncological treatment of advanced UC for the foreseeable future.

Despite the rapidly evolving treatment landscape for patients with mUC, there is still a need to further improve existing and develop new chemotherapy-based treatment regimens. The novel combination of VG, explored in the randomised phase II trial VINGEM (**Paper III**), shows promise as first-line treatment in mUC patients with impaired renal function, and it has a notably high ORR. This combination merits further evaluation in mUC patients and might also be of interest to investigate as neoadjuvant treatment in cisplatin-ineligible MIBC patients with renal impairment, a group that currently lacks treatment options. In the future, it is possible that mUC patients who are ineligible for cisplatin can achieve OS similar to that reached in cisplatin-eligible patients by applying new treatment options such as VG or combinations of chemotherapy and drugs with different mechanisms of action in individualised treatment strategies. Recently, drugs targeting angiogenesis and the VEGF pathway have been explored in mUC [139, 141, 151]. The combination of vinflunine and the antiangiogenic multi-tyrosine kinase inhibitor sorafenib can be safely administered, as shown in the VINSOR trial (**Paper II**). The next step is to further evaluate this novel combination in a phase II trial to assess efficacy and survival compared to standard second-line treatment in mUC. In accordance with this approach, other trials targeting angiogenesis in combination with chemotherapy or ICIs are in progress and may be an effective treatment alternative for selected patients.

As the number of available treatment options increases in mUC, there is an urgent need to develop biomarker-driven precision medicine approaches to optimise treatment selection and sequencing for the individual patient. For cisplatin, promising treatment-predictive biomarkers are evolving, such as DDR genes and molecular classification [107], although no validated markers have yet been established for routine clinical use. Considering vinflunine, no biomarkers have been investigated in the clinical setting. Blood samples have been collected from patients in the VINGEM trial (**Paper III**) to enable future studies on biomarker

profiling aimed at exploring molecules involved in the cytotoxic mechanisms of action for vinca alkaloids [153, 154].

In patients with MIBC, neoadjuvant cisplatin-based chemotherapy is undoubtedly effective in achieving pathological downstaging of the primary tumour in the bladder (**Paper IV**), although approximately one third of the patients do relapse. Initial small single-arm studies with ICIs in the neoadjuvant setting show promising results [51, 52], and a large number of ongoing trials are evaluating ICIs and targeted drugs in monotherapy or in combination with chemotherapy in patients with MIBC [5, 155]. Patients with remaining residual tumour in the bladder after neoadjuvant chemotherapy constitute a high-risk population with poor prognosis. Future research should focus on additional adjuvant systemic therapy after neoadjuvant chemotherapy and cystectomy for selected patients [156-158], preferably considering drugs with different mechanisms of action and based on the molecular profile of the residual tumour in the cystectomy specimen. Furthermore, neoadjuvant chemotherapy presently in use excludes patients that are cisplatin-ineligible, and the current standard of care is cystectomy alone. In cisplatin-unfit patients with MIBC, ICIs are also being explored [34, 155], although other potential treatment options should be investigated as well, for example, using vinflunine together with gemcitabine (**Paper III**) or vinflunine as the backbone in combinations with non-nephrotoxic drugs with different mechanisms of actions.

The neoadjuvant setting for patients with MIBC is suitable for evaluating signals of activity with new agents and novel treatment combinations by assessing the tumour response in the cystectomy specimen. In addition, longitudinal tumour sampling from pre-treatment TUR-B and post-treatment cystectomy is easily accessible, which enables analyses of treatment-induced molecular changes that may serve as biomarkers. There are currently no available biomarkers to distinguish MIBC patients that are *de novo* resistant to cisplatin treatment. Moreover, approximately 10% of patients that achieve complete response do relapse, indicating possible heterogeneity in the sensitivity of tumour cells to cisplatin in the primary tumour in the bladder and in circulating micro-metastases. Future neoadjuvant studies should include a precision medicine approach aimed at selecting patients likely to benefit from neoadjuvant cisplatin-combination chemotherapy based on molecular profiling.

Advanced UC is a disease associated with high morbidity, and especially patients in late stage mUC often present with multiple tumour-associated symptoms and overall poor PS. Furthermore, the toxicity profiles of the chemotherapy regimens available today are all to different extents associated with more or less severe side effects. In addition to the development of more effective treatment options, future clinical trials should focus not only on measurement of efficacy endpoints like response and survival, but also on more direct evidence of clinical benefit for the patients, such as relief of tumour-related symptoms, increased physical functioning, and improved HRQoL, for this severely ill patient population.

## 8 POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Cancer i urinblåsan är den sjunde vanligaste cancerformen i Sverige med drygt 3000 fall per år. Sjukdomen delas in i tre stadier: 1) yttlig urinblåsecancer som begränsar sig till inne i urinblåsan och har god prognos, 2) avancerad sjukdom som går djupare in i muskellagret i urinblåsan (muskelinvasiv sjukdom) och 3) metastaserad sjukdom där tumören har spridit sig ut i kroppen, vanligtvis till lymfkörtlar, lever, lungor eller skelettet. Muskelinvasiv och metastaserad urinblåsecancer är aggressiva sjukdomar som kan ge upphov till många tumörrelaterade symtom och har hög dödlighet. För patienter med metastaserad sjukdom är överlevnaden i medeltal endast cirka 15 månader. Behovet av att utveckla nya effektivare behandlingsalternativ är således mycket stort.

Standardbehandling för muskelinvasiv urinblåsecancer är cytostatika ("cellgifter") följt av bortoperation av urinblåsan. Trots intensiv behandling så drabbas knappt hälften av patienterna av återfall i form av antingen lokal tumörväxt i lilla bäckenet eller av metastaser, varvid sjukdomen inte längre är botbar. För patienter med metastaserad urinblåsecancer är bromsande behandling med platinum-innehållande cytostatikakombinationer (cisplatin eller karboplatin) standardbehandling sedan slutet av 80-talet. Vinflunine introducerades 2009 som det första nya läkemedlet på flera decennier, och tillför en begränsad men viktig överlevnadsvinst för patienter som inte längre har effekt av platinumbehandling. Immunterapi har medfört ett stort genombrott i behandlingen av många cancertyper, och så även för urinblåsecancer där immunterapi godkändes 2017 för behandling av patienter med metastaserad sjukdom.

Syftet med denna doktorsavhandling var att förbättra behandlingen med cytostatika i olika faser av urinblåsecancer, med avseende på dess effekt på tumören, biverkningsprofil och hälsorelaterad livskvalitet. Vinflunine undersöktes som singel-behandling och i nya behandlingskombinationer vid metastaserad sjukdom. Vidare utvärderades två olika cellgiftskombinationer vid muskelinvasiv sjukdom.

I **Studie I** analyserades användandet av den nyligen introducerade cytostatikan vinflunine i klinisk rutinsjukvård genom att studera tumöreffekt, behandlingsmönster och biverkningar. Vinflunine var effektivt i klinisk rutin och förlängde överlevnaden i samma storleksordning som i den originalstudie som låg till grund för godkännandet av vinflunine. Patienter med dåligt allmäntillstånd hade kortare överlevnad och drabbades av fler biverkningar än patienter med bra allmäntillstånd.

I **Studie II** studerades vinflunine i en ny kombination med det målstyrda läkemedlet sorafenib i en nordisk läkemedelsstudie för patienter med metastaserad urinblåsecancer som sviktat på tidigare platinumbaserad cytostatikabehandling. Då denna nya behandlingskombination aldrig getts till patienter tidigare var studiens huvudsakliga mål att analysera biverkningsprofilen och bestämma vilka doser av de två ingående läkemedlen som var

lämpliga att ge. Vinflunine tillsammans med sorafenib kunde ges på ett säkert sätt och biverkningarna var i linje med vad som tidigare är beskrivet för vart och ett av läkemedlen. Denna nya behandlingskombination är lovande och bör undersökas i ytterligare studier med fokus på dess tumörkrympande effekt och överlevnad hos patienter med metastaserad urinblåsecancer.

I **Studie III** undersöktes vinflunine i en ny kombination med cytostatikan gemcitabine hos patienter med metastaserad urinblåsecancer i en stor nordisk läkemedelsstudie. Patienterna lottades till antingen behandling med den nya experimentella behandlingen eller till dagens standardbehandling, som utgörs av cytostatikakombinationen gemcitabine och karboplatin. Vinflunine tillsammans med gemcitabine ledde inte längre överlevnad jämfört med dagens standardbehandling, men däremot minskade storleken av tumörerna i markant högre grad hos patienter som erhöll den experimentella behandlingen. Biverkningsprofilen var acceptabel för den nya kombinationen, även om en högre andel av patienterna drabbades av låga vita blodkroppar och till följd av detta infektioner. Trots skillnaderna i biverkningsprofilen uppmättes den hälsorelaterad livskvalitet som likvärdig mellan de två behandlingsgrupperna. Med tanke på att kombination vinflunine och gemcitabine minskade tumörstorleken mycket effektivt vore det intressant att undersöka denna nya kombination i ytterligare studier vid muskelinvasiv och metastaserad urinblåsecancer.

I **Studie IV** jämfördes behandling mellan två olika scheman av cytostatikakombinationen gemcitabine och cisplatin hos patienter med muskelinvasiv sjukdom innan bortoperation av urinblåsan. Patienter som erhöll behandling med ett mer dosintensivt 3-veckors schema erhöll en bättre krympande effekt på tumören i urinblåsan, dock även med fler biverkningar, än patienter som erhöll ett 4-veckors schema. Trots skillnad i effekt på tumören i urinblåsan kunde ingen skillnad ses i andelen patienter som drabbades av återfall eller dog av sjukdomen mellan de två olika behandlingsschemana.

Sammanfattningsvis bidrar denna doktorsavhandling med ny kunskap om effekten och biverkningsprofilen av vinflunine som singelbehandling och i nya lovande behandlingskombinationer hos patienter med metastaserad urinblåsecancer, samt ny kunskap om den tumörkrympande effekten av cytostatikakombinationen gemcitabine och cisplatin hos patienter med muskelinvasiv sjukdom. De senaste åren har utvecklingen av behandlingsalternativ vid metastaserad urinblåsecancer gått mycket snabbt. Förutom immunterapi som redan etablerat sig som en av standardbehandlingarna i klinisk rutin, är flertalet andra läkemedel, främst så kallade målriktade läkemedel, på väg att godkännas inom kort. Metastaserad urinblåsecancer är en sjukdom med ofta mycket svåra cancerrelaterade symtom och trots nya behandlingsalternativ är överlevnaden begränsad. Det är därför av största vikt att utveckla nya behandlingar som både förlänger livet och förbättrar patienternas hälsorelaterade livskvalitet.



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# 11 APPENDICES

## APPENDIX A

### Participating centres associated with the NUCOG collaboration

	Paper I	Paper II	Paper III	Paper IV
<b>Sweden</b>				
Karolinska Universitetssjukhuset, Stockholm	x	x	x	x
Norrlands Universitetssjukhus, Umeå			x	
Sahlgrenska Universitetssjukhus, Göteborg			x	
Skånes Universitetssjukhus, Malmö/Lund			x	
Västmanlands Sjukhus, Västerås			x	
<b>Denmark</b>				
Aalborg Universitetshospital, Aalborg			x	x
Aarhus Universitetshospital, Aarhus		x	x	x
Herlev Gentofte Hospital, Herlev			x	x
Odense Universitetshospital, Odense	x		x	x
Rigshospitalet, Copenhagen	x	x	x	x
<b>Finland</b>				
Åbo Universitetscentralsjukhus, Åbo			x	

## APPENDIX B

### Patients treated with vinflunine in the Nordic countries: A retrospective study

Date of registration: \_\_\_\_\_ Name of registrant: \_\_\_\_\_

Participating centre: \_\_\_\_\_

#### 1. GENERAL INFORMATION (at time of diagnosis, at least muscle-invasive disease)

Gender: Male ☐ Female ☐

Date of diagnosis: \_\_\_\_\_

Age at diagnosis: \_\_\_\_\_

Primary tumour location: Bladder ☐ Urethra ☐ Ureter ☐ Renal pelvis ☐ Urachus ☐ Other ☐ \_\_\_\_\_

Histology: Transitional cell carcinoma ☐ Adenocarcinoma ☐ Urachal adenocarcinoma ☐  
Small cell carcinoma ☐ Squamous cell carcinoma ☐ Sarcomatoid carcinoma ☐  
TCC with neuroendocrine features ☐ TCC with squamous differentiation ☐  
TCC with glandular differentiation ☐ TCC with sarcomatoid differentiation ☐  
TCC with micropapillary features ☐ Pure micropapillary ☐ Other ☐

Clinical Stage at diagnosis: T2a–T4b: \_\_\_\_\_ N0–N3: \_\_\_\_\_ M0–M1: \_\_\_\_\_

#### 2. PRIMARY TREATMENT

Surgical removal of primary tumour (cystectomy): Yes ☐ No ☐

Radiation therapy to primary tumour: Yes ☐ No ☐

Concurrent Chemotherapy with radiation therapy: Yes ☐ No ☐ Chemotherapy regimen: \_\_\_\_\_

#### PERIOPERATIVE SYSTEMIC THERAPY

Neoadjuvant therapy: Yes ☐ No ☐ Chemotherapy regimen: \_\_\_\_\_

Adjuvant therapy? Yes ☐ No ☐ Chemotherapy regimen: \_\_\_\_\_

#### 3. METASTATIC DISEASE/RECURRENCE

Date of diagnosis of first metastatic or locally recurrent disease: \_\_\_\_\_

Metastatic sites: Local recurrence bladder ☐  
Lymph nodes ☐ Liver ☐ Lung ☐ Bone ☐ Brain ☐ Other ☐ \_\_\_\_\_

#### 4. SYSTEMIC THERAPY (prior to vinflunine therapy)

##### FIRST-LINE SYSTEMIC THERAPY

Was first-line chemotherapy administered? Yes ☐ No ☐

First-line cisplatin-based regimen: \_\_\_\_\_ Number of Cycles: \_\_\_\_\_

Reason for discontinuation: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

First-line carboplatin-based regimen: \_\_\_\_\_ Number of Cycles: \_\_\_\_\_

Reason for carboplatin-based regimen: Impaired renal function ☐ Performance status > 1 ☐  
Comorbidity ☐ Other ☐ \_\_\_\_\_

Reason for discontinuation: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

First-line single gemcitabine regimen: \_\_\_\_\_ Number of Cycles: \_\_\_\_\_

Reason for single gemcitabine regimen: Impaired renal function ☐ Performance status > 1 ☐  
Comorbidity ☐ Other ☐ \_\_\_\_\_

Reason for discontinuation: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

Other first-line chemotherapy regimen: \_\_\_\_\_ Number of cycles: \_\_\_\_\_  
Reason for other chemotherapy regimen: Impaired renal function ☐ Performance status > 1 ☐  
Comorbidity ☐ Other ☐ \_\_\_\_\_  
Reason for discontinuation: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

## SECOND-LINE SYSTEMIC THERAPY

Was second-line chemotherapy administered? Yes ☐ No ☐

Second-line chemotherapy regimen: \_\_\_\_\_ Number of cycles: \_\_\_\_\_  
Reason for discontinuation: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

## THIRD-LINE SYSTEMIC THERAPY

Was third-line chemotherapy administered? Yes ☐ No ☐

Third-line chemotherapy regimen: \_\_\_\_\_ Number of cycles: \_\_\_\_\_  
Reason for discontinuation: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

## PLATINUM RESISTANCE

Relapse or progression within 6 months after carboplatin or cisplatin-based chemotherapy: Yes ☐ No ☐

---

## 5. BASE-LINE PARAMETERS VINFLUNINE TREATMENT

Age: \_\_\_\_\_ ECOG PS: \_\_\_\_\_ Prior pelvic/abdominal irradiation: Yes ☐ No ☐  
GFR (iohexol or Cr-EDTA clearance, ml/min): \_\_\_\_\_  
Alkaline phosphatase : Normal ☐ Elevated ☐ Haemoglobin: < 100 ☐ ≥ 100  
Metastatic sites: Local recurrence bladder ☐  
Lymph nodes ☐ Liver ☐ Lung ☐ Bone ☐ Brain ☐ Other ☐ \_\_\_\_\_  
Relevant comorbidity: \_\_\_\_\_

---

## 6. VINFLUNINE TREATMENT

Vinflunine given as: first line ☐ second line ☐ third line ☐ fourth line ☐

Initial dose of vinflunine: 320 mg/m<sup>2</sup> ☐ 280 mg/m<sup>2</sup> ☐ 250 mg/m<sup>2</sup> ☐

Number of cycles: \_\_\_\_\_

Date of starting vinflunine: \_\_\_\_\_ Date of discontinuing vinflunine: \_\_\_\_\_

Dose reduction: Yes ☐ No ☐

Dose delay (≥ 1 week): Yes ☐ No ☐

Reason for dose reduction and/or dose delay: Related to the vinflunine treatment ☐  
Related to the bladder cancer disease ☐  
Other ☐ \_\_\_\_\_

Re-challenge of vinflunine: Yes ☐ No ☐

Length of pause (weeks since previous vinflunine treatment period was discontinued): \_\_\_\_\_

Dose of vinflunine when re-challenged: 320 mg/m<sup>2</sup> ☐ 280 mg/m<sup>2</sup> ☐ 250 mg/m<sup>2</sup> ☐

Number of cycles when re-challenged: \_\_\_\_\_

Date of starting vinflunine: \_\_\_\_\_ Date of discontinuing vinflunine: \_\_\_\_\_

Dose reduction when re-challenged: Yes ☐ No ☐

Dose delay when re-challenged ( $\geq 1$  week): Yes ☐ No ☐

Reason for dose reduction and/or dose delay when re-challenged: Related to the vinflunine treatment ☐  
Related to the bladder cancer disease ☐  
Other ☐ \_\_\_\_\_

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## 7. TERMINATION OF VINFLUNINE TREATMENT

Total number of cycles: \_\_\_\_\_

Reason for discontinuing: Progressive disease ☐ Toxicity ☐ Other ☐ \_\_\_\_\_

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## 8. OTHER ONCOLOGICAL TREATMENTS (during vinflunine treatment)

Palliative radiotherapy: Yes ☐ No ☐ Bisphosphonates: Yes ☐ No ☐ Other: \_\_\_\_\_

---

## 9. PAIN

Pain at baseline (when initiating vinflunine): Yes ☐ No ☐

Pain reduction during/after vinflunine: Yes ☐ No ☐

---

## 10. TREATMENT-RELATED TOXICITY (grade 3/4 AEs related to vinflunine treatment)

Anaemia: Yes ☐ No ☐ Neutropenia: Yes ☐ No ☐ Febrile neutropenia: Yes ☐ No ☐  
Thrombocytopenia: Yes ☐ No ☐

Fatigue: Yes ☐ No ☐ Nausea: Yes ☐ No ☐ Vomiting: Yes ☐ No ☐ Stomatitis/Mucositis: Yes ☐ No ☐

Abdominal pain: Yes ☐ No ☐ Constipation: Yes ☐ No ☐

Myalgia: Yes ☐ No ☐ Neuropathy sensory: Yes ☐ No ☐

Alopecia: Yes ☐ No ☐ Infusion/Injection site reaction: Yes ☐ No ☐

---

## 11. EFFICACY RESULTS (of vinflunine treatment)

Evaluation no. 1 Total number of cycles: \_\_\_\_\_  
Complete response: Yes ☐ No ☐  
Partial response: Yes ☐ No ☐  
Stable disease: Yes ☐ No ☐  
Progress: Yes ☐ No ☐

Evaluation no. 2 Total number of cycles: \_\_\_\_\_  
Complete response: Yes ☐ No ☐  
Partial response: Yes ☐ No ☐  
Stable disease: Yes ☐ No ☐  
Progress: Yes ☐ No ☐

Evaluation no. 3 Total number of cycles: \_\_\_\_\_  
Complete response: Yes ☐ No ☐  
Partial response: Yes ☐ No ☐  
Stable disease: Yes ☐ No ☐  
Progress: Yes ☐ No ☐

Evaluation no. 4 Total number of cycles: \_\_\_\_\_  
Complete response: Yes ☐ No ☐  
Partial response: Yes ☐ No ☐  
Stable disease: Yes ☐ No ☐  
Progress: Yes ☐ No ☐

Evaluation no. 5 Total number of cycles: \_\_\_\_\_  
Complete response: Yes ☐ No ☐  
Partial response: Yes ☐ No ☐  
Stable disease: Yes ☐ No ☐  
Progress: Yes ☐ No ☐

Evaluation no. 6 Total number of cycles: \_\_\_\_\_  
Complete response: Yes ☐ No ☐  
Partial response: Yes ☐ No ☐  
Stable disease: Yes ☐ No ☐  
Progress: Yes ☐ No ☐

Duration of response = Complete response and/or partial response (weeks): \_\_\_\_\_

Progression-free survival (PFS) = Complete response and/or partial response and/or stable disease (weeks): \_\_\_\_\_

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## 12. SURVIVAL STATUS

Status at last follow-up: Alive ☐ Dead ☐ Date of death: \_\_\_\_\_



## **APPENDIX C**

### **VARIABLE TEMPLATE NUCOG V**

#### **3- vs 4-week GC as neoadjuvant treatment in muscle invasive bladder cancer**

All dates are registered as **yyyy-mm-dd!**

#### **BASELINE PARAMETERS**

1. Patient study ID
2. Age at start of neoadjuvant chemotherapy
3. Sex
4. ECOG at start of neoadjuvant (0 or 1)
5. cTNM at start of neoadjuvant
6. GFR at start (iohexole clearance [uncorrected] or chrome-EDTA if available, otherwise calculated GFR)

#### **TREATMENT PATTERNS**

7. Date of first cycle of neoadjuvant chemotherapy
8. Date of last given neoadjuvant chemotherapy (date of last infusion of cisplatin or gemcitabine)
9. Total number of cycles (despite dose reductions, dose delays, omitted doses, etc.)
10. Number of cycles with d 8 given
11. Reason if d 8 not given, more than one reason possible (more than one cycle with omitted d 8 or more than one reason for omitting d 8 at the same cycle):  
anaemia = 1; neutropenia = 2; thrombocytopenia = 3; neutropenic fever = 4;  
infection = 5; decreased kidney function = 6; other reason = 7.
12. Number of cycles with reduction of cisplatin dose
13. Reason for cisplatin dose reduction, more than one reason possible (more than one cycle with dose reduction or more than one reason for dose reduction during the same cycle):  
anaemia = 1; neutropenia = 2; thrombocytopenia = 3; neutropenic fever = 4;  
infection = 5; decreased kidney function = 6; other reason = 7.
14. Number of cycles with reduction of gemcitabine dose
15. Reason for gemcitabine dose reduction, more than one reason possible (more than one cycle with dose reduction or more than one reason for dose reduction during the same cycle):  
anaemia = 1; neutropenia = 2; thrombocytopenia = 3; neutropenic fever = 4;  
infection = 5; decreased kidney function = 6; other reason = 7.
16. Number of cycles with dose delay
17. Reason for dose delay (5 days or more), more than one reason possible (more than one cycle with dose delay or more than one reason for dose delay during the same cycle):  
anaemia = 1; neutropenia = 2; thrombocytopenia = 3; neutropenic fever = 4;  
infection = 5; decreased kidney function = 6; other reason = 7.
18. Number of cycles with C-GSF

19. Terminated treatment prematurely (yes/no)

If yes, type the reason for premature termination, more than one reason possible:

anaemia = 1; neutropenia = 2; thrombocytopenia = 3; neutropenic fever = 4;  
infection = 5; decreased kidney function = 6; thromboembolic event = 7; hearing  
loss = 8; peripheral neuropathy = 9; heart failure = 10; progressive disease = 11;  
other reason = 12.

**TOXICITY** Register toxicity if it appears at least once in the same patient. Do NOT register single blood values!

20. Anaemia grade 3/4 (haemoglobin < 4.9 mmol/L or < 80 g/L) (yes/no)

21. Neutropenia grade 3/4 (neutrophils < 1.0 x 10<sup>9</sup> /L) (yes/no)

22. Febrile neutropenia (neutrophils < 1.0 x 10<sup>9</sup> /L and a single temperature of 38.5 degrees or sustained temperature of ≥ 38 degrees for more than 1 hour) (yes/no)

23. Thrombocytopenia grade 3/4 (thrombocytes < 50 x 10<sup>9</sup> /L) (yes/no)

24. Non-haematological AE (NH-AE) grade 3/4 (yes/no)

If yes, type of NH-AE:

infection = 1; thromboembolic event = 2; decreased kidney function = 3; hearing  
loss = 4; peripheral neuropathy = 5; heart failure = 6; other reason = 7.

25. Death related to neoadjuvant treatment (yes/no)

**OUTCOME MEASUREMENT**

26. Date of cystectomy

27. pT (pT0, pT1, pT2, pT3, pT4, pTIS, pTa)

28. pN (pN0, pN1, pN2, pN3)

29. Relapse (yes/no)

30. Date of relapse (date of radiology, biopsy, or obvious clinical progression)

31. Death (yes/no)

32. Death due to bladder cancer (yes/no)

33. Date of death

34. Date of last follow-up (for patients still alive)

## APPENDIX D



### EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

Your birthdate (Day, Month, Year):

Today's date (Day, Month, Year):

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a <u>long</u> walk?	1	2	3	4
3. Do you have any trouble taking a <u>short</u> walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

#### During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
	1	2	3	4
17. Have you had diarrhea?				
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your <u>family</u> life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your <u>social</u> activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

29. How would you rate your overall health during the past week?

30. How would you rate your overall quality of life during the past week?

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## APPENDIX E

### Ethical and medical approvals

Paper	Regulatory authority	Reference number	Title	Approval date
<b>I</b>	Regionala Etik-prövningsnämnden, Stockholm	Dnr: 2013/664-31/3	Patienter behandlade med cytostatika vid avancerad blåscancer – en retrospektiv kvalitetssäkringsanalys	02-05-2013
	Denmark		Letter from professor H. Pappot concerning the ethical approval, Rigshospitalet, Copenhagen	05-02-2020
<b>II</b>	Regionala Etik-prövningsnämnden, Stockholm	Dnr: 2011/1398-31/1	Tidig klinisk prövning av tolerabilitet samt analys av biomarkörer vid behandling av avancerad cancer utgången från urinvägarna med standardcytostatika vinflunine (Javlor®) med tillägg av cancerläkemedlet sorafenib (Nexavar®)	12-10-2011
	Läkemedelsverket	151:2012/12127	Klinisk prövning av Javlor	05-03-2012
	Center for Sundhed, De Videnskabetiske Komiteer i Region Hovedstaden, Copenhagen	Protokol nr.: H-1-2012-079	”Tidlig klinisk afprøvning af tolerabilitet ved behandling af metastatisk kræft udgående fra urinvejene med en ny kombination: standardcytostatika vinflunin med tillæg af kræftlægemidlet sorafenib”	04-09-2012
	Sundhedsstyrelsen, Denmark	Journalnr: 2012053960	“An Exploratory Phase I Study with Sorafenib in Addition to Vinflunine in Progressive Locally Advanced or Metastatic Transitional Cell Carcinoma of the Urothelial Tract”	02-11-2012
<b>III</b>	VHP, the European Medicines Agency	VHP 201362	A multicentre, randomised phase II trial of vinflunine/gemcitabine versus carboplatin /gemcitabine as first-line treatment in patients with metastatic urothelial carcinoma unfit for cisplatin-based chemotherapy due to impaired renal function.	20-08-2013
	VHP, the European Medicines Agency	VHP 201362/SA1	Amendment	22-03-2016
	Regionala Etik-prövningsnämnden, Stockholm	Dnr: 2013/1238-31/2	En jämförande studie mellan två olika cytostatikabehandlingar för patienter med spridd blåscancer och nedsatt njurfunktion	21-08-2013
	Regionala Etik-prövningsnämnden, Stockholm	Dnr: 2016/669-32	Substantial amendment concerning the VINGEM trial	23-05-2016
	Läkemedelsverket, Sverige	Dnr: 5.1-2013-57539	Klinisk prövning av Javlor (NUCOGI-VINGEM)	10-09-2013
	Läkemedelsverket, Sverige	Dnr: 5.1-2016-14161	Väsentlig ändring: Klinisk prövning av Javlor (NUCOGI-VINGEM)	08-04-2016
	Center for Sundhed, De Videnskabetiske Komiteer i Region Hovedstaden, Copenhagen	Protokol nr: H-2-2013-106	”En multicenter randomiseret fase II undersøgelse af vinflunin/gemcitabin versus carboplatin/gemcitabin som 1.linje behandling til patienter med metastatisk kræft udgående fr urinvejene og nedsat nyrefunktion”	04-02-2014
	Center for Sundhed, De Videnskabetiske Komiteer i Region Hovedstaden, Copenhagen	Ref.: H-2-2013-106 Anm.nr. 55764	Tillægsprotokoll amendment til protokollen VINGEM	16-11-2016
	Sundhedsstyrelsen, Denmark	Journalnr: 2013083932	“A multicentre, randomised phase II trial of vinflunine/gemcitabine versus carboplatin/gemcitabine as first-line treatment in patients with metastatic urothelial carcinoma unfit for cisplatin-based chemotherapy due to impaired renal function.” Protokol: VINGEM, EudoraCT-nummer: 2013-002417-35	05-09-2013
	Sundhedsstyrelsen, Denmark	Case no: 2016040217	Amendment Protokol: VINGEM, EudoraCT-nummer: 2013-002417-35	07-04-2016
	Eettinen toimikunta, Varsinais-Suomen sairaanhoitopiirin kuntayhtymä, Åbo	ETMK Dnro: 175/1801/2015	”Vinflunine/gemcitabiini ensilinjan yhdistelmähoitona virtsarakon syöpää sairastavilla postilailla, joilla on munuaisten vajaatoimintaa	15-12-2015

			eivätkä siten sovellu sisplatiinipohjaiseen lääkehoitoon NUCOG 1 – VINGEM”	
	Eettinen toimikunta, Varsinais-Suomen sairaanhoitopiirin kuntayhtymä, Åbo	ETMK Dnro: 175/1801/2015	Amendment	21-06-2016
	Finnish Medicines Agency	KLnro: 154/2015	A multicentre, randomised phase II trial of vinflunine/gemcitabine versus carboplatin/gemcitabine as first-line treatment in patients with metastatic urothelial carcinoma unfit for cisplatin-based chemotherapy due to impaired renal function. NUCOGI-VINGEM	10-12-2015
	Finnish Medicines Agency		Amendment	
<b>IV</b>	Regionala Etik-prövningsnämnden, Stockholm	Dnr: 2013/664-31/3	Patienter behandlade med cytostatika vid avancerad blåscancer – en retrospektiv kvalitetssäkringsanalys	02-05-2013
	Regionala Etik-prövningsnämnden, Stockholm	Dnr: 2016/1089-32	Amendment till Etikgodkännande Dnr 2013/664-31/3	07-06-2016
	Etikprövningsmyndigheten, Sverige	Dnr: 2020-00616	Amendment till Etikgodkännande Dnr 2013/664-31/3 och Amendment Dnr 2016/1089-32	04-03-2020
	Styrelsen for patientsikkerhed, Copenhagen	Sagsnr. 3-3013-3078/1	Godkendelse af ansøgning om videregivelse af oplysninger fra patient-journaler til brug for forskningsprojekt Ansøgning STPS neoadjuverende projekt	17-07-2019